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VARIATION AND PHYSIOLOGIC SPECIALIZATION IN THE COMMON SCAB FUNGUS (ACTINOMYCES SCABIES)[1]

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INTRODUCTION

The common scab fungus (*Actinomyces scabies* (Thaxt.) Güss.) is found in the soil of millions of acres of the best potato-growing sections of the United States, and is a source of serious economic loss to growers. Seed and soil treatments have been devised, but these are expensive and often unsatisfactory. The most effective means of controlling scab is to breed varieties that are resistant to scab. This method frequently involves the expenditure of considerable time and money, but the results when once obtained appear to be permanent.

Clark, Stevenson, and Schaal (*3*)[2] and Krantz and Eide (*6*) have shown that there are wide differences among varieties and even among seedlings in their reaction to scab. Some are nearly immune, others completely susceptible. It has also been shown that immunity and susceptibility are transmissible to the progeny.

In breeding new varieties that combine scab resistance with high, yield and good cooking and market qualities it has been found that a variety which is resistant to scab in one locality may be less resistant in another. In a series of tests made in a number of potato scab gardens in different parts of the United States (*14*), it was found that the variability was due in part to environmental conditions and in part to physiologic races of *Actinomyces scabies*. That *A. scabies* may comprise different strains was considered by Millard and Burr (*10*), and the existence of races was established by Leach, Decker, and Becker (*7*). The present work was undertaken to study (1) the occurrence of physiologic races of *A. scabies* and their relation to the variable behavior of scab, (2) the factors affecting the development of races or strains, and (3) the necessity for testing the reaction of potato varieties to the various parasitic strains of the scab organism. The studies were started at the Aroostook Farm, Presque Isle, Maine, and were continued at the Plant Industry Station, Beltsville, Md., and later at University Farm, St. Paul, Minn.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 186.

METHODS OF ISOLATION AND DESIGNATION OF CULTURES

The method of isolating *Actinomyces scabies* from deep pustules on the tubers of potatoes (*Solanum tuberosum* L.) was a slight modification of that described by Shapovalov (*13*). An infected tuber was washed, the pustules were wiped lightly with cotton saturated with 95 percent alcohol, and the alcohol on the surface was burned. The corky covering was then lifted with a sterile scalpel, and the underlying tuber tissue was transferred to a tube of melted agar; transfers were made to other tubes of melted agar, which were poured into Petri dishes in order to make it easier to obtain colonies of *A. scabies* free from other fungi and bacteria. When isolates were made from shallow or superficial scab spots the whole scabby area was flamed lightly, macerated, and transferred to melted agar. Dilution plates were then poured, as described above. In most cases colonies of *A. scabies* appeared after 48 hours.

The scab organism was isolated from soil by pouring dilution plates from 10-gm. soil samples.

Single-cell isolates were obtained by using a micromanipulator with a research microscope. With the end of a needle a small quantity of aerial mycelium was tapped lightly over a sterile cover slip; on the surface of this were deposited a number of single cells. The cover slip was then inverted over a modified Van Tieghem cell for examination under the microscope. By means of a glass rod drawn to a diameter of 1μ or 2μ single cells were picked up and transferred to an agar drop on a cover slip. The cover slip was then inverted over a Van Tieghem cell containing sterile water and placed in an incubator. If the cell germinated, it was transferred to an agar slant from which subcultures were made later.

The following system was used to designate the cultures: M1, M2, etc., are single-cell cultures; S1, S2, etc., are sectors from such cultures. For example, 147M1S2 is sector 2 of single-cell culture M1 of parent isolate 147. Single-cell cultures from sector cultures were in turn indicated by M1, M2, etc.; for example, 147M6S3M1.

SOURCE AND GENERAL CHARACTERISTICS OF ISOLATES

Waksman (*15*) described a number of species of *Actinomyces* on the basis of cultural characters, but he did not give a definite specific name to the strains found on potatoes. Lutman and Cunningham (*8*) concluded that all known strains were able to produce a brown pigmentation on media containing proteins. The isolates of *Actinomyces scabies* listed in table 1 were obtained from scab pustules on potato tubers grown in various States. Scab cultures were also obtained from Ireland and Australia. In the early studies it was noted that sometimes several culturally different isolates of *A. scabies* could be obtained from a single pustule. Cultures from these colonies differed in growth type and in the pigmentation that they produced on a given medium. Usually isolates of a given cultural type were consistently obtained from tubers grown in a certain area or on a certain soil type.

Table 2 lists the colors of the mycelium and of the pigment produced by 33 cultures when grown on the modified potato-dextrose agar designated as medium 1 in table 3. All cultures listed in table 2 were found to be pathogenic, but they varied in degree of virulence, as indicated in table 1.

TABLE 1.—*Source of isolates and cultures of Actinomyces scabies, type of pustule, and most susceptible of tested potato varieties*

Isolate or culture No.	Source of infected tuber or culture	Pustule type [1] on—		Soil type on which naturally infected tuber grew	Most susceptible variety tested
		Naturally infected tuber	Inoculated tuber		
J11M1	Minnesota	1	R	Mineral	Katahdin.
23M1	Maine	1	1	do	Green Mountain.
66M1	do	2	2	do	Do.
104 [2,3]	Ireland		1		Do.
105 [2]	Australia		1		Do.
108M1	Minnesota	2	2	Peat	Irish Cobbler.
110M1	do	1	1	do	Do.
115M1	do	1	2	Mineral	Do.
117M2	Colorado	1	R	do	Katahdin. [4]
118M4	Minnesota	1	1	do	Green Mountain.
119M1	Colorado	1	1	do	Katahdin.
124M2	Minnesota	1	R	Peat	U. S. D. A. seedling.
125M1	do	1	1	do	Green Mountain.
126M2	do	1	1	do	Do.
133M1	Colorado	1	1	Mineral	Do.
136M3	Minnesota	1	1	Peat	Do.
143M1	Colorado	1	1	Mineral	Katahdin.
147M2	Minnesota	2	2	do	Do.
153M1	do	1	1	do	Green Mountain.
166M1	Colorado	R	R	do	U. S. D. A. seedling.
176M4	Wyoming	1	1	do	Green Mountain.
181M2	do	1	1	do	Do.
183M1	do	1	2	do	Do.
185M1	Minnesota	2	2	Peat	U. S. D. A. seedling.
200M1	Colorado	3	R	Mineral	Do.
205M3	Minnesota	1	1	do	Do.
210M1	do	1	1	do	Katahdin.
220M2	do	1	2	do	U. S. D. A. seedling.
223M2	do	1	1	Peat	Green Mountain.
234M1	Maine	1	1	Mineral	Do.
242M3	California	1	1	do	Do.
250M1	Minnesota	1	1	Peat	Irish Cobbler.
252M3	do	2	1	do	Do.

[1] 1, susceptible; 2, moderately susceptible; 3, resistant; R, russeting caused by scab organism (skin checked and rough).
[2] Not single-spored.
[3] From T. N. Greeves, Queens University, Belfast.
[4] Only variety tested.

TABLE 2.—*Color characteristics of thirty-three 6-week-old pathogenic isolates and cultures of Actinomyces scabies grown on modified potato-dextrose agar*

Isolate or culture No.	Mycelium	Pigment in agar	Isolate or culture No.	Mycelium	Pigment in agar
J11M1	Yellow	Deep yellow.	143M1	Blue gray	Dark brown.
23M1	Gray	None.	147M2	Light blue	Deep blue.
66M1	do	Do.	153M1	Green gray	Brown.
104 [1]	Green	Blue gray.	166M1	Blue gray	None.
105 [1]	Gray	None.	176M4	Light blue	Violet to red.
108M1	Light gray	Do.	181M2	Dark gray	Dark brown.
110M1	Blue gray	Brown.	183M1	Light gray	Brown.
115M1	Dark gray	Do.	185M1	Blue gray	Pink to red.
117M2	Buff [2]	Light brown.	200M1	Dark gray	Brown.
118M4	Dark gray	Dark brown.	205M3	Buff	Light brown.
119M1	do	None.	210M1	Gray	None.
124M2	do	Pink, turning blue as culture aged.	220M2	Red [3]	Dark red.
			223M2	White	None.
125M1	Light gray	Brown.	234M1	Gray	Light brown.
126M2	Blue gray	None.	242M3	Dark gray	Brown.
133M1	Dark gray	Dark brown.	250M1	Light gray	None.
136M3	Light gray	Do.	252M3	do	Brown.

[1] Not single-spored. [2] Aerial. [3] Cheesy.

TABLE 3.—*Media used in studying cultural characters of Actinomyces scabies*

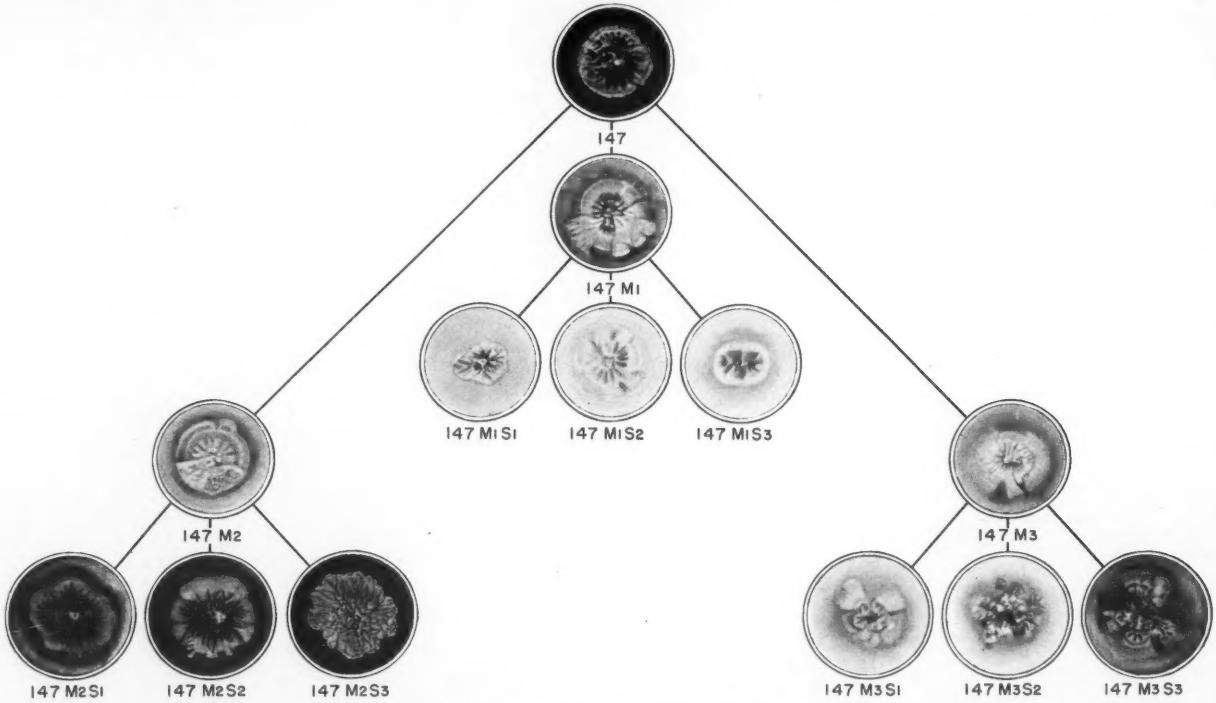
Medium			pH after sterilization
No.	Name	Formula	
1	Modified potato-dextrose agar.	Peeled potatoes, 300 gm Dextrose, 5 gm Agar, 20 gm Tap water, 1,000 cc Adjusted to pH 6.8	6.8 to 7.0
2	Egg-albumen agar (Waksman).	Dextrose, 10 gm KH_2PO_4, 0.5 gm $MgSO_4$, 0.2 gm $Fe_2(SO_4)_3$, trace Egg albumen, 0.15 gm Agar, 20.0 gm Distilled water, 1,000 cc Adjusted to pH 7.0	6.5 to 7.0
3	Bacto-nutrient agar	Dehydrated Bacto-nutrient agar, 23 gm Distilled water, 1,000 cc Adjusted to pH 7.0	6.8
4	Richards' agar (modified).	KNO_3, 1 gm KH_2PO_4, 0.5 gm $MgSO_4$, 0.2 gm $FeCl_3$, trace Sucrose, 5.0 gm Agar, 20.0 gm Distilled water, 1,000 cc Adjusted to pH 7.0	6.8 to 7.0
5	Richards' agar (modified) plus thiamine.	Same as 4 with 0.3 gm. thiamine added Adjusted to pH 7.0	6.8 to 7.0
6	Richards' agar (modified).	$MgSO_4$, 1.0 gm Urea, 2.0 gm KH_2PO_4, 1.0 gm $FeCl_3$, trace Agar, 20.0 gm Distilled water, 1,000 cc Adjusted to pH 7.0	6.8 to 7.0
7	Potato-dextrose agar	Same as 1 except that whole tubers of a potato variety resistant to scab were used as a source of potato juice.	6.8 to 7.0
8	do	Same as 7 except that potato juice was from whole tubers of a scab-susceptible variety (Irish Cobbler).	6.8 to 7.0

CULTURAL VARIATION

FREQUENCY OF SECTORING

Sectoring in *Actinomyces scabies*, which was first observed in 1-month-old or older cultures in flasks, has not previously been reported in the literature. Several culturally different strains produced sectors that differed greatly from one another. Culture 147M1, isolated from a pustule on a tuber grown at Castle Danger, Minn., produced many sectors during 4 weeks in culture. In some cases as many as nine sectors appeared in a single colony; these varied in type and rate of mycelial growth and in color of pigment. Other cultures sectored less frequently.

Plate 1 shows diagrammatically the relation of the culturally different strains 147M1, 147M2, and 147M3, which originated as single cells from the parent isolate 147. The parent isolate 147 (pl. 1) had a deep-blue pigment, but only one of the single cells isolated from it produced the deep-blue pigment or lighter shades of blue. Culture 147M1 developed sectors. Cultures from three of these failed to produce the deep-blue pigment in the medium. After 3 months cultures from eight sectors of 147M1 had produced sectors, and a culture from one sector had failed to produce sectors. Apparently this sector was stable since further culturing in media of various types and with different pH values failed to induce sectoring. Although it might have



Variants produced by *Actinomyces scabies* isolate 147.

been possible to obtain several more sectors from each culture, cultures were not made from all sectors. Several of the sectors shown in cultures 147M3S2 and 147M3S3 were cultured later, and these in turn formed sectors. This process could have been repeated, and many culturally different strains of *Actinomyces scabies* could probably have been produced.

Figure 1 shows diagrammatically how sectoring occurred in isolate 66. In this case, too, some of the variants sectored. Neither the parent nor the variates produced pigment in the medium. Sectoring of the type found in certain cultures of *Actinomyces scabies* might account for the many strains of the fungus that have been found in soils and on potato tubers.

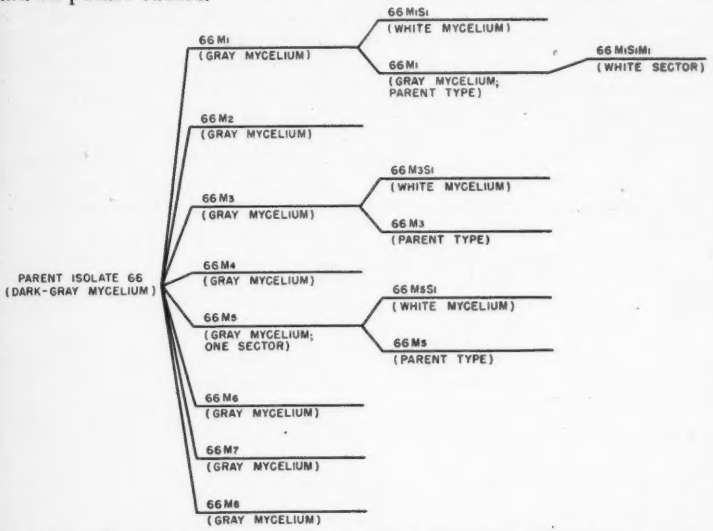


FIGURE 1.—Diagrammatic relation of the sectors produced by three of eight single-cell cultures originating from parent isolate 66.

MORPHOLOGY OF VARIOUS ISOLATES

Studies were made to determine how culturally different isolates would vary morphologically. In these studies mycelial cells rather than spores were used for measurement, since some cultures did not produce any aerial spores or spirals on any medium used. The size of mycelial cells and the presence or absence of spirals on the aerial mycelium were noted.

Drechsler (*4*) found that many species of *Actinomyces* could be differentiated on the basis of morphology, that the presence or absence of spirals constituted a differential character, that some of the spirals were sinistrorse and others dextrorse, and that there were noticeable differences in the sizes of mycelial cells of the several species listed.

The mean sizes of cells in various cultures differed. The cells of the spirals were sometimes twice as large as the mycelial cells, and several cultures produced larger cells on the tips of the spirals. Some investigators speak of these terminal cells as spores.

Table 4 gives the mean sizes of mycelial cells and records the presence or absence of spirals in 20 cultures of *Actinomyces scabies* grown on modified potato-dextrose, egg-albumen, and Bacto-nutrient agars. The cells used for the measurements were taken near the center of the colony, and the means of 100 cells were obtained. Six of the cultures did not produce spirals on any of the media; 14 produced sinistrorse or dextrorse spirals in varying abundance. Both the type of spiral and the direction of the turns appeared to vary with the culture. Culture 166M2 produced abundant aerial mycelium without spirals, and culture 220M1 produced a slimy growth without spirals. Culture 23M1 produced spirals, whereas 23M1S1M1, a single-cell culture from a sector from 23M1, did not produce spirals. There was very little variation in the sizes of cells in most cultures. The parent culture 23M1 had mycelial cells 1μ by 2μ, and those of its variant measured 1μ by 1μ. This difference in cell size indicates that when sectoring occurs in *A. scabies* the morphology of the variant may differ from that of the parent.

Attempts to demonstrate the presence of nuclei in *Actinomyces scabies* failed. Feulgen's stain was found best for staining the cells, but even with this, nuclei could not be recognized with certainty. There may, however, be numerous nuclei in a single cell, for the cell contents under high magnification appear to contain many dark-staining, minute objects.

TABLE 4.—*Cell sizes and presence or absence of spirals in cultures of Actinomyces scabies on different media*[1]

Culture No.	Medium 1 (modified potato-dextrose agar)		Medium 2 (egg-albumen agar)		Medium 3 (Bacto-nutrient agar)	
	Spirals present (+) or absent (−)	Mean size of cells	Spirals present (+) or absent (−)	Mean size of cells	Spirals present (+) or absent (−)	Mean size of cells
		μ		μ		μ
23M1	+	1×2	+	1×2	+	1×2
23M1S1M1	−	1×1	−	1×1	−	1×1
66M1	+	1.5×2	+	1.5×2	+	1.5×2
104M1	+	1.5×2	+	1.5×2	+	1.5×2
105M1	+	1×1	+	1×1	+	1×1
119M1	−	1.5×2	−	1.5×2	−	1.5×2
133M1	+	1×2	+	1×2	+	1×2
136M4	+	2×2	+	1.5×2	+	2×2
147M1	+	2×2	+	2×2	+	2×2
147M1S1	+	2×2	+	2×2	+	2×2
147M6S3M1	+	1×2	+	1×2	+	1×2
153M1	+	1.5×2	+	1.5×2	+	1.5×2
157M1	+	2×2	+	1.5×2	+	1.5×2
166M2	−	1×1	−	1×1	−	1×1
176M4	+	1.5×2	+	1.5×2.5	+	1.5×2
181M1	+	1.5×2	+	1.5×2	+	1.5×2
200M1	−	2×2	−	2×2	−	2×2
220M1	−	1×1.5	−	1×1.5	−	1×1
234M1	+	1.5×1.5	+	1.5×1.5	+	1.5×1.5
250M2	−	1×2	−	1×2	−	1×2

[1] See table 3 for formulas.

FACTORS AFFECTING GROWTH AND SECTORING

EFFECTS OF NUTRIENTS

The effects of different nutrients on the rate of growth and the sectoring of *Actinomyces scabies* were studied by using the synthetic media 2, 3, 4, 5, and 6 described in table 3. All these media were

adjusted to pH 7.0 before sterilization; after sterilization the pH values varied from 6.5 to 7.0. Barss (*2*) called attention to the variable composition of media made from plant or animal tissue and the difficulty of using such media to study rate and character of growth. This difficulty is especially great in a study of the scab organism, and for that reason synthetic media were used.

Ten different cultures of *Actinomyces scabies* were grown in duplicate on the five synthetic and modified potato-dextrose media in 250-cc. Erlenmeyer flasks at approximately 20° C. for 8 weeks. The mycelial growth was more rapid on the synthetic media than on the potato-dextrose agar adjusted to the same pH value, but aerial mycelia were sparse and could be seen only by careful observation. The most rapid growth was made on medium 5, which contained 0.3 mg. of thiamine per liter. Best growth of aerial mycelium was noted on medium 3. A medium having a high nitrogen content inhibited aerial mycelium and limited the size of the colonies. Afanasiev (*1*) found that high nitrogen inhibited production of aerial mycelium by parasitic races of *A. scabies* but did not affect the saprophytes; this finding partly substantiates the present results.

After 6 weeks only four cultures showed sectors, but after 8 weeks all had produced them. At the end of 8 weeks the greatest number of sectors had been produced on medium 5. The stimulation of the rate of mycelial growth and of the production of sectors by the addition of thiamine agrees with the results of Oyama (*11*), who found that the vitamin B complex increased growth in the Dermatocytes.

Effects of Temperature

Cultures of four strains of *Actinomyces* isolated from tubers from three States, representing different mean seasonable temperatures, were grown for 56 days on modified potato-dextrose agar adjusted to pH 6.8 at 18°, 20°, 22°, and 24° C.

The differences in temperature tolerance of these four cultures were not great, as the growth curves of figure 2 show. All the cultures grew readily at temperatures from 18° to 24° C. As the colonies increased beyond a diameter of 60 mm. the rate of growth became slower. Regardless of how long a culture was grown in a 250-cc. flask containing 75 cc. of medium, the diameter of the colony never exceeded 65 of the 75 mm. of surface diameter available.

No consistent differences appeared in the rate of sectoring and the type and number of sectors produced by the four cultures grown at 18°, 20°, 22°, and 24° C. over a period of 12 weeks. In several duplicate cultures sectors developing at 22° and 24° were almost identical in appearance, whereas at 18° and 20° differences in number and type of sectors were more evident; at 18° sectors were present in several of the cultures, but they were smaller than those in cultures grown at 22° and 24°.

Effects of Hydrogen-ion Concentration

It has been commonly accepted that *Actinomyces scabies* will not grow well on acid media and that scab infection does not occur on potato tubers growing in soil with a pH value less than 5. However, cases have been reported (*12*) where serious scab has occurred in soil of pH 5.4.

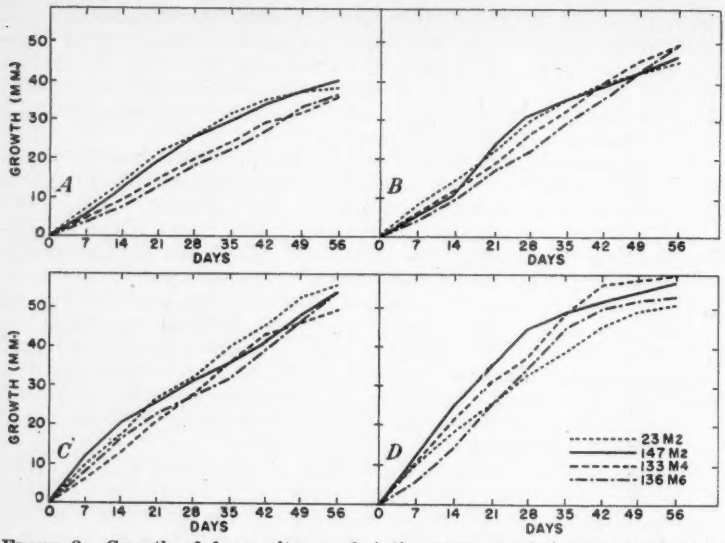


FIGURE 2.—Growth of four cultures of *Actinomyces scabies* on potato-dextrose agar adjusted to pH 6.8: *A*, 18°; *B*, 20°; *C*, 22°; and *D*, 24° C.

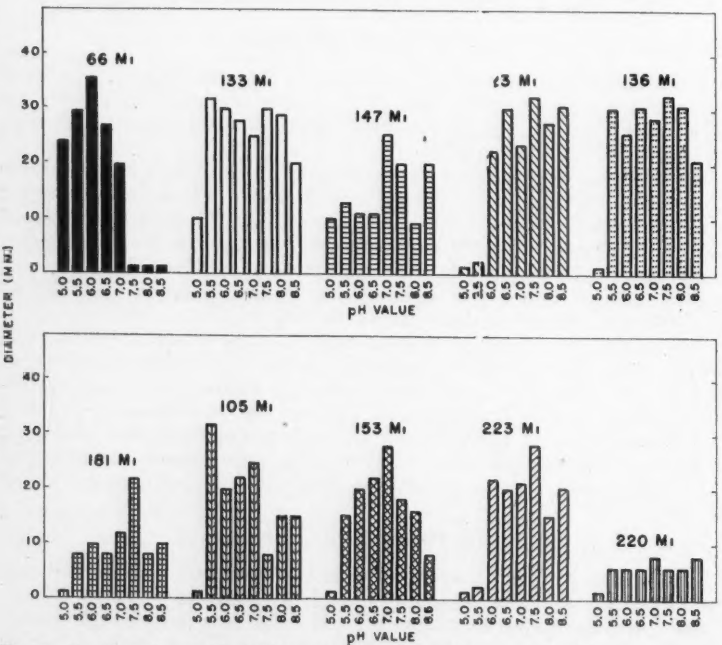


FIGURE 3.—Growth of 10 *Actinomyces scabies* cultures after 7 days on potato-dextrose agar adjusted to different pH values.

Ten cultures of *Actinomyces scabies* obtained from different potato-growing areas were grown on modified potato-dextrose agar adjusted to 8 different pH values from 5.0 to 8.5 in duplicate 250-cc. flasks containing 75 cc. of medium. All flasks were inoculated uniformly by placing a drop of sterile water containing a high concentration of spores in the center of the medium and were incubated at room temperature (approximately 22° C.). The diameters of the colonies were measured at the end of 7 and 60 days. Figures 3 and 4 show the relative growths of the 10 cultures.

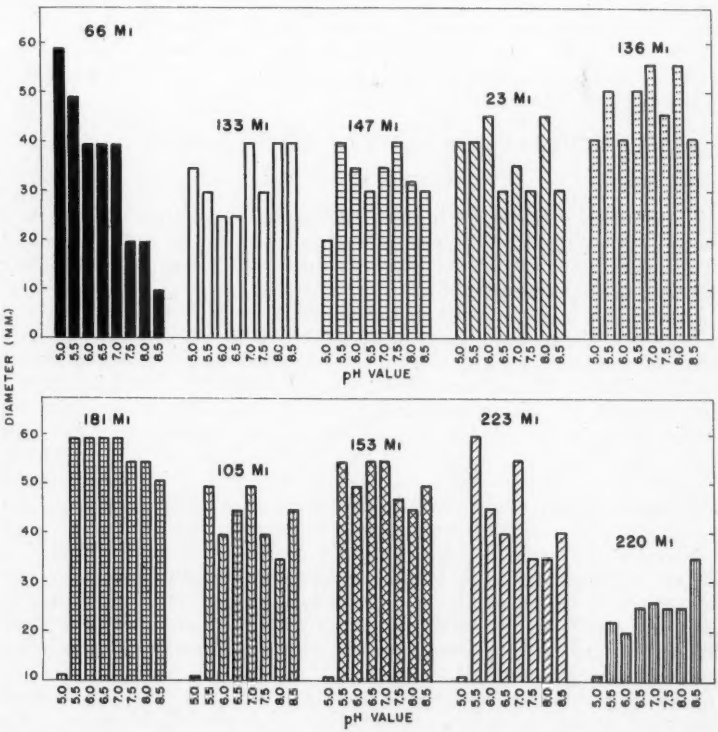


FIGURE 4.—Growth of 10 *Actinomyces scabies* cultures after 60 days on potato-dextrose agar adjusted to different pH values.

After 7 days seven of the cultures showed very little growth at pH 5, but four of these seven developed rapidly at pH 5.5, indicating that their pH tolerance was between 5.0 and 5.5 (fig. 3). Culture 220M1 grew slowly on all media; and there was little difference in its rate of growth regardless of the pH of the medium. Maximum growth occurred at pH 8.5 (fig. 4), but subsequent tests with this culture showed that it did not grow more even on more alkaline media.

These observations indicate that many strains of *Actinomyces scabies* grow best at approximately pH 7.0 and do poorly below 5.5.

This finding agrees with observations on the effect of the hydrogen-ion concentration of the soil on scab severity. At the end of 60 days, however, culture 66M1 had made its maximum growth at pH 5.0 (fig. 4). This culture, which was isolated from a tuber grown in soil where scab was severe, could not be distinguished culturally or morphologically from certain others, but it could be differentiated by its tolerance to acidity and its parasitism on certain potato varieties (*12*).

While these cultures were growing in flasks, observations were also made on the effect of different hydrogen-ion concentrations on sectoring. At the end of 40 days very few sectors had appeared, but at the end of 60 days sectors were developing in one or both of the duplicate flasks that showed appreciable growth. Sectors were noted at all pH values above 5.0, but no single type predominated. Variation in *Actinomyces scabies* apparently is not greatly affected by the reaction of the supporting medium.

GREENHOUSE TESTS OF PATHOGENICITY

METHODS OF TESTING

The method used to distinguish parasitically different strains consisted in growing different varieties of potatoes in the greenhouse and inoculating the soil with cultures of *Actinomyces scabies* immediately after the tubers were planted.

In the inoculation studies in the greenhouse at Beltsville, Md., and at St. Paul, Minn., seed pieces were treated with a 1 to 1,000 mercuric chloride solution for 1½ hours, then washed under running water for 10 minutes, planted in soil with a pH favorable for the development of scab, and steam-sterilized for 10 hours at 10 pounds' pressure. The inoculum was added to the soil as a water suspension of the scab organism. The concentration of the inoculum was kept as uniform as possible for the several tests. When a large amount of sterilized soil was inoculated and placed in the greenhouse bench, the inoculum was added with a sprinkling can as the soil was being mixed.

When large quantities of inoculum were needed a spore suspension was made in sterile distilled water and several drops were spread over the surface of agar medium in Petri dishes. After considerable aerial mycelium had been produced it was scraped off with a sterile scalpel and placed in sterile tap water; measured quantities of this suspension were then added to the soil.

All pot inoculations were made in duplicate 6-inch pots, which were separated on the bench by panes of glass high enough to prevent the mixing of cultures by splashing when the pots were watered. Soil moisture was kept as uniform as possible. Preliminary tests showed that ordinary tap water did not contain *Actinomyces;* hence it was not necessary to use sterile water. All plants were allowed to mature, and notes were taken when the tubers were dug.

Pustule type was considered the most important factor in determining the degree of parasitism of the different isolates. The number of pustules produced on a tuber was not considered as important as whether the infection was deep, shallow, or russeted. In all cases there were sufficient pustules of a given type on the tubers to make accurate readings possible. A modification of the system of estimating scab infection employed by Clark, Stevenson. and Schaal (*3*) was

used; according to this system 1 indicates susceptibility and 3 resistance. This system was found to give a satisfactory differentiation between the types of infection. The type of scab that caused a normally smooth variety of potato to look russeted and scurfy was called russeting, as distinguished from the pustule type.

The commercial and seedling varieties of potatoes tested to determine whether the various isolates constitute physiological races of the scab organism are listed in table 5. Commercial varieties are designated by names and seedling varieties by numbers.

TABLE 5.—*Reaction of certain commercial varieties and seedlings of potatoes grown in a greenhouse to single-cell cultures of Actinomyces scabies*

Culture No.	Source of infected tuber or culture	Pustule type [1] produced on tubers of—							
		Katahdin	Green Mountain	Seedling 1037–5	Seedling 295–12	Seedling 627–213	Seedling 528–212	Hindenburg	Seedling 2621
M5M1	Michigan [2]	0	3	0	0	0	R	3	0
J11M1	Minnesota	0	0	0	0	0	0	0	0
23M1	Maine	1	1	2	2	1	2	3	3
23M1S1	do	2	1	2	2	2	2	0	0
66M1	do	2	2	1	3	2	3	0	3
66M1S1	do	2	R	R	0	0	R	0	0
104M1	Ireland	3	1	2	0	0	3	0	0
105M1	Australia	1	1	0	0	0	0	0	0
110M1	Minnesota	1	1	1	2	------	2	3	------
118M1	do	3	1	2	3	0	3	------	------
119M1	Colorado	1	2	0	3	R	R	------	------
124M1	Minnesota	0	R	0	R	------	R	------	------
128M1	Colorado	3	R	2	0	0	0	0	------
133M1	do	1	1	1	2	3	------	------	3
136M1	Minnesota	1	1	1	2	0	3	0	0
143M1	Colorado	1	1	2	1	3	2	0	------
147M1	Minnesota	0	2	1	R	------	3	0	1
153M1	do	3	1	1	0	0	0	0	1
157M1	Colorado	1	1	1	2	------	0	3	------
166M1	do	2	3	R	0	0	3	0	0
170M1	do	0	0	3	0	3	3	0	------
176M1	Wyoming	0	1	2	0	0	0	0	0
178M1	do	0	1	2	0	2	R	0	------
181M1	do	1	1	1	1	2	2	3	1
183M1	do	3	1	1	0	------	3	3	0
194M1	Colorado	1	0	1	0	0	0	0	0
200M1	do	1	1	2	0	0	2	0	0
205M1	Minnesota	2	0	1	2	2	2	0	3
210M1	do	1	1	3	R	0	3	0	1
217M1	do	1	1	1	R	3	2	0	1
220M1	do	0	0	0	2	0	0	0	0
223M1	do	1	1	1	3	1	3	0	1
231M1	do	0	1	0	0	3	0	0	2
232M1	do	0	3	0	0	0	3	0	3
236M1	California	2	1	2	R	0	2	0	0
242M1	do	0	1	3	3	0	0	0	0
250M1	Minnesota	0	1	2	0	0	0	0	0
252M1	do	2	1	1	R	0	3	0	1
3031M1	New Jersey [3]	0	3	2	------	0	0	------	------
3352M1	do [3]	0	3	2	2	0	0	------	------

[1] 0, no scab; 1, susceptible; 2, moderately susceptible; 3. resistant; R, russeting caused by scab organism (skin checked and rough).
[2] From J. H. Muncie, Michigan Agricultural Experiment Station.
[3] From New Jersey Agricultural Experiment Station.

RELATIVE PATHOGENICITY OF VARIOUS CULTURES

Table 5 summarizes the results of 2 inoculation tests to determine the scab reaction of 3 commercial and 5 seedling varieties of potatoes selected for their differential reaction, as noted in field experiments, to 40 cultures of *Actinomyces scabies*. All but 1 of the cultures were found to be pathogenic in varying degrees, and even this was parasitic

in other tests. These experiments show that when a single-cell culture is used shortly after isolation from a tuber as inoculum for tubers grown in sterilized soil, it produces a distinct pustule type on a given variety of potato. In the field more than 1 type of pustule may be found on a tuber. This suggests the presence of more than 1 parasitic strain in the soil. The scab reactions of 2 commercial and 4 seedling varieties to 11 isolates are shown in figure 5. Plainly these isolates differed in pathogenicity as well as in cultural and physiological characters.

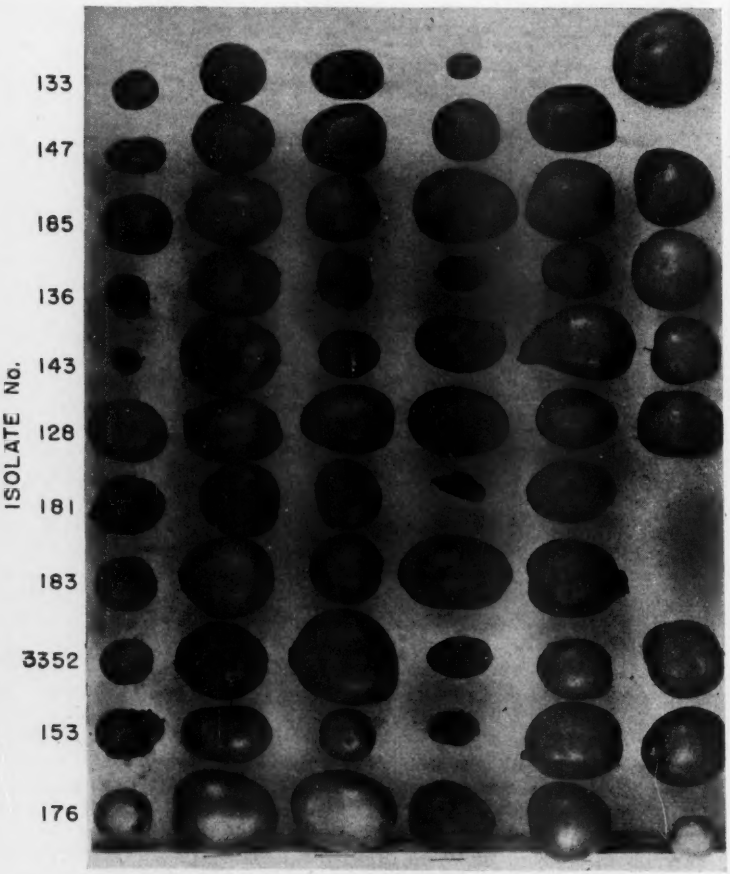


FIGURE 5.—Representative types of scab infection produced by 11 isolates of *Actinomyces scabies* (designated by numbers at left) on commercial and seedling varieties of potatoes in greenhouse inoculation tests: *A*, Green Mountain; *B*, seedling 295–12; *C*, Katahdin; *D*, seedling 1037–5; *E*, seedling 528–212; and *F*, seedling 627–213.

RELATIVE PATHOGENICITY OF VARIANT AND PARENT CULTURES

The occurrence of cultural variants suggested that differences in pathogenicity might exist. Consequently, tests were made to see whether such differences really occurred. Cultures 23M1S1 and 66M1S1 were culturally different from their parent cultures. Pathogenicity tests of these variants and the parent cultures showed slight differences in the type of pustules produced on some of the varieties (table 5). These variants were less virulent than the parent cultures on some varieties. For example, culture 66M1S1 produced only russeting-type scab on Green Mountain, whereas the parent culture (66M1) produced type 2 pustules. Parent culture 66M1 was more parasitic than the variant on all varieties tested except Katahdin and Hindenburg. Parent culture 23M1 was more parasitic than the variant 23M1S1 on Katahdin, Hindenburg, and seedling varieties 627–213 and 2621. On the other varieties tested this variant produced pustules similar to those caused by the parent culture. The results of these limited tests indicate that the variants produced by parasitic cultures of *Actinomyces scabies* may differ in pathogenicity from the parent cultures.

RELATION OF VARIOUS ISOLATES TO RUSSETING

Russeting of normally smooth-skinned varieties of potatoes has been attributed to various causes. Many growers are of the opinion that it is caused by physical and chemical conditions of the soil, such

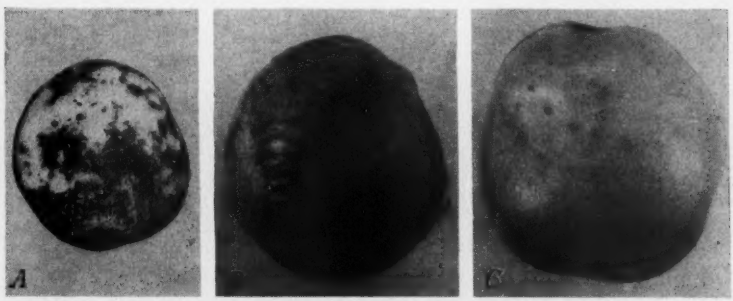


FIGURE 6.—*A*, Russeting type of scab on a Green Mountain tuber grown in Maine; *B*, russeting on Green Mountain tuber inoculated with *Actinomyces* 124M1; *C*, Green Mountain tuber grown in uninoculated soil.

as high acidity or high alkalinity, or by fertilizer burn. Lutman, Livingston, and Schmidt (*9*) suggested that pathogenicity might be reduced if the organism existed for a time in the absence of the potato and that it would then produce a russeting or milder form of scab. Goss (*5*) suggested that russeting in Green Mountain under field conditions may be caused by a different species of *Actinomyces*.

The data presented in table 5 show that one culture, 124M1, when it caused infection, produced russeting only. Another, 166M1, produced only shallow type 2 or 3 pustules or russeting on the varieties that became infected. Isolate 166 was from a tuber showing russet-type infection. Of the 40 cultures listed in table 5, 12 produced russeting on 1 or more of the varieties tested, some of which are normally smooth. Figure 6, *B*, shows typical russeting produced in

greenhouse inoculations by culture 124M1. Reisolates from this type of scab appeared identical with the inoculum. Russeting of the type shown in figure 6, *B*, can be caused by certain strains of *Actinomyces scabies* on normally smooth-skinned potato tubers.

VARIETAL REACTION TO SCAB IN DIFFERENT LOCALITIES

In breeding potatoes for resistance to scab and various other diseases the possibility of physiologic specialization of the pathogen must be considered. In uniform scab test plots in various potato-growing

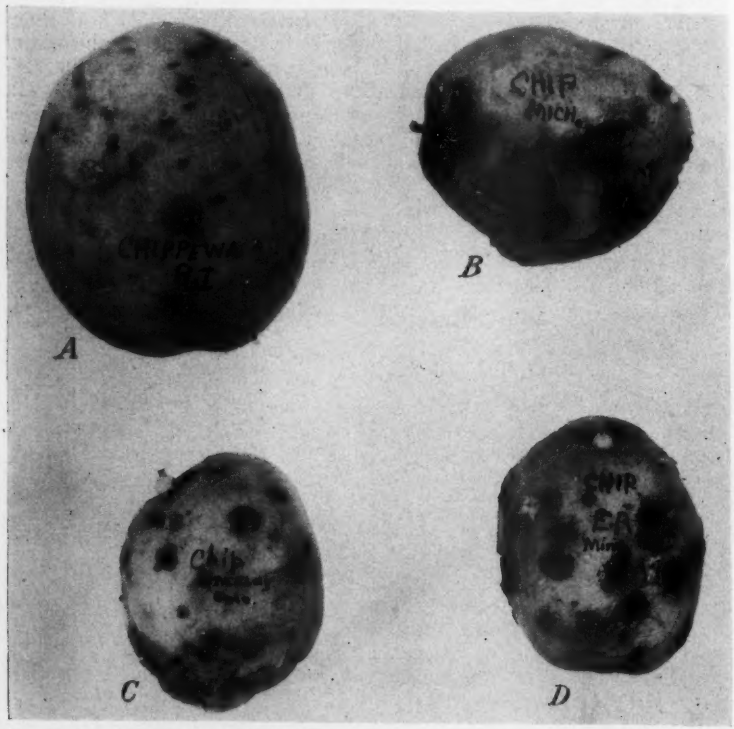


FIGURE 7.—Types of scab pustules on Chippewa tubers produced in scab test plots in Rhode Island (*A*); Michigan (*B*); Colorado (*C*); and Minnesota (*D*). In Michigan and Minnesota the plots were on peat soil; in Rhode Island and Colorado on mineral soil.

areas it has been found that a given variety may differ greatly in its reaction to scab in different localities (figs. 7 to 10). The pronounced differences in the types of pustules indicate, as the greenhouse tests did, that different strains or races of *Actinomyces scabies* may be involved.

DISCUSSION

In these studies an attempt was made to determine the existence and possible importance of strains and physiologic races of *Actinomyces*

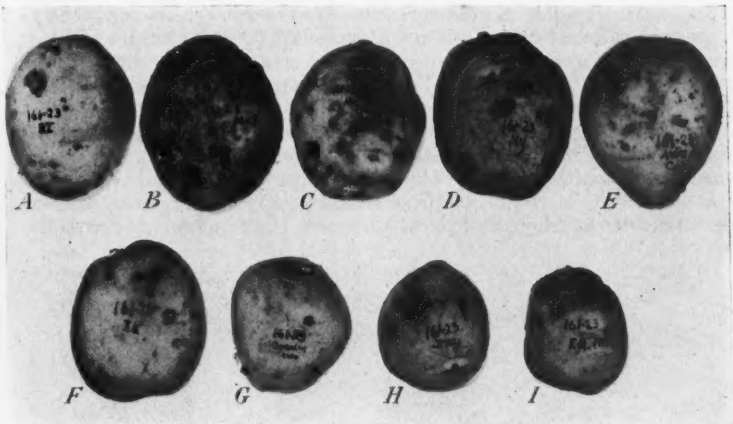


FIGURE 8.—Types of scab pustules on tubers of United States Department of Agriculture seedling 161–23 produced in scab test plots in Rhode Island (*A*); Michigan (*B*); Maine (*C*); New York (*D*); Avon, Colo. (*E*); Iowa (*F*); Greeley, Colo. (*G*); Indiana (*H*); and Minnesota (*I*). In Iowa, Michigan, and Minnesota the plots were on peat soil; in the other States on mineral soil.

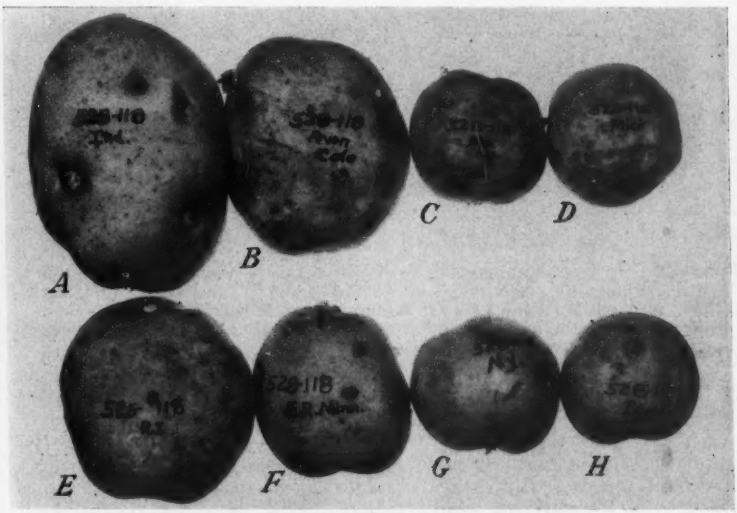


FIGURE 9.—Type of scab pustules on tubers of United States Department of Agriculture seedling 528–118 produced in scab test plots in Indiana (*A*); Colorado (*B*); Maine (*C*); Michigan (*D*); Rhode Island (*E*); Minnesota (*F*); New York (*G*); and Iowa (*H*). This seedling was resistant to scab in these eight States.

scabies. That strains do exist has been well established by these studies. From a collection of scabby tubers from different potato-growing areas it was possible to obtain isolates of *A. scabies* differing greatly in type and color of mycelium and in color of pigment produced in the medium. It seemed possible that these strains might differ also in morphological characters and degree of pathogenicity, as experiments by other investigators had suggested that parasitic strains of *A. scabies* might have certain cultural characters in common and that it might be possible to distinguish parasitic and saprophytic types by simpler and easier methods than by inoculation of tubers, which is always a laborious and slow method. In these studies, however, no correlation was detected between degree of parasitism and degree of color of mycelium

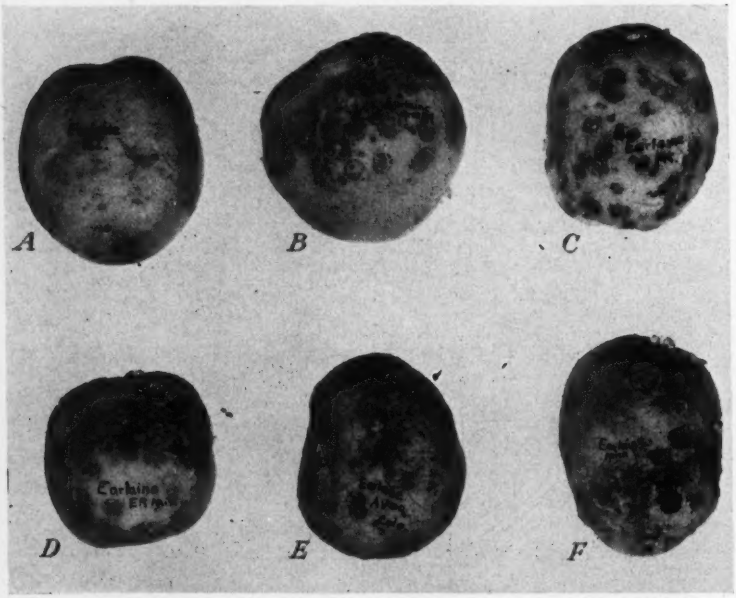


FIGURE 10.—Types of scab pustules on Earlaine tubers produced in scab plots in Rhode Island (*A*); Greeley, Colo. (*B*); Maine (*C*); Minnesota (*D*); Avon, Colo. (*E*); and Michigan (*F*). In Minnesota and Michigan, plots were on peat soil; in the other States on mineral soil. The Avon, Colo., plot was located in an intermountain valley at an altitude of 7,500 feet.

or medium. There were no morphological characters of *A. scabies* that distinguished parasitic from nonparasitic strains. The production of spirals on the aerial mycelium appears to be constant for a given strain, and the medium on which the culture was grown did not affect this characteristic. Both spiral- and nonspiral-producing strains were found to be parasitic on potato tubers; the direction of the turns in the spirals was not correlated with pathogenicity. The size of mycelial cells did not serve as a distinguishing character. In short, inoculation of potato tubers seems to be the only method for determining the relative pathogenicity of strains of *A. scabies*.

The large number of strains of *Actinomyces scabies* found in soils suggests that variation is occurring in the soil, and that possibly some of these new strains may be more pathogenic than others on the tubers of a given variety of potatoes. Thus a single variety may possibly vary from highly resistant to completely susceptible in the same area, depending on the pathogenicity of the variants present. How rapidly new variants are produced in nature is not known. Some isolates produced very few sectors in cultures; others produced many. Nutrients, temperature, and the hydrogen-ion concentration of the medium did not appear to have much effect on rate of sectoring, which appears to be racial in character.

Most investigators agree that infection by the scab organism is influenced by the reaction of the soil. It is assumed that *Actinomyces* does not infect potato tubers growing in soil at pH 5.0 or below, but that it does infect those growing in soils at pH 6.0 to 7.5. These experiments indicate that certain strains of *Actinomyces scabies* are capable of causing scab on tubers growing in soil with a pH value as low as 5.4.

Field studies showed that some seedling potatoes might be resistant in one section of the country and susceptible in another (figs. 7 to 10), and greenhouse tests indicated that different varieties differed in reaction to the various isolates (table 5).

Although these investigations indicate that scab resistance in potatoes is relatively stable, as is shown by the consistency of pustules on a given variety in a given environment, it is clear that the reaction of a variety may vary with the strain of the pathogen. Much remains to be done on the nature of resistance. Some varieties may have morphological resistance and others physiological resistance. More reliable information on the effect of the various strains of *Actinomyces scabies* on resistance will have to be determined in future studies.

SUMMARY

Single-cell cultures of *Actinomyces scabies* were obtained from potatoes grown on various soil types in different States. They differed in color of mycelium and of pigment produced on modified potato-dextrose agar.

The isolates were unstable and produced variants that were often culturally different from the parents and from each other. One variant studied did not sector during a 3-month period.

No isolate produced cells that averaged more than 2.5 μ long. Some isolates produced spirals, and others did not.

Aerial mycelium was inhibited by high-nitrogen media. Most rapid growth was made on a medium containing thiamine. On such a medium the number of sectors was greater.

On potato-dextrose agar four isolates grew somewhat better at 20° to 24° C. than at 18°. Sectors were produced at all temperatures studied, but those in cultures at 18° were smaller than those at 22° and 24°.

Ten isolates grew on potato-dextrose agar adjusted to pH 5 to 8.5; after 60 days five of these had made very little growth in the medium at pH 5. Sectors were produced at all pH values except 5.

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A number of isolates tested on three commercial and five seedling varieties of potatoes differed in pathogenicity. No definite correlation was detected between pathogenicity and cultural or other characteristics. Variants seemed to differ from their parent cultures in pathogenicity. Certain strains produced russeting of normally smooth-skinned varieties.

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ACQUIRED IMMUNITY FROM CURLY TOP IN TOBACCO AND TOMATO[1]

By JAMES M. WALLACE

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INTRODUCTION

Since Wingard (*24*) [2] reported that tobacco plants that had recovered from severe stages of the ring spot disease failed to show the usual disease symptoms when reinoculated with the ring spot virus, similar reactions have been observed in several other plant viruses, including curly top (*4, 12, 21*). Price (*15*) has presented an extensive and detailed review of this subject.

The term "recovery" is used in this paper to designate a permanent change in the plants from a severely diseased to a mildly diseased condition without total loss of the causative virus; and the recovery dealt with in these investigations is distinct from a simple masking of symptoms, which results from such factors as environment and natural resistance. With some viruses and under certain conditions, recovery is of such a high degree that it is difficult to distinguish between the recovered plants and the disease-free plants. In other instances the recovered plants continue to show mild symptoms but do not at any time revert permanently to a diseased condition as severe as that before recovery. In all instances where it has been demonstrated that such recovered plants and their vegetative progeny are resistant to injury from reinoculation with the virus of the disease from which they recovered, it also has been shown that the plants continually harbor virus. Although the concentration of virus in the recovered plants may be lower than that in nonrecovered plants, there is obviously some multiplication of virus since it is still present in vegetatively propagated plants many generations removed from the plant in which recovery originally occurred (*13, 14, 21*).

Investigations of recovery from curly top (*21, 22, 23*) have revealed some reactions not previously obtained in similar studies of recovery from other virus diseases of plants. First, it was demonstrated that the acquired immunity from curly top could be transferred from recovered to healthy tobacco (*Nicotiana tabacum* L.) plants by grafting. Later, it was shown that tomato (*Lycopersicon esculentum* Mill.) plants of varieties that commonly have no power of recovery could be "immunized" by grafting with recovered tobacco plants. Further study showed that in plants that had the power of recovery the process of "immunization" took place gradually but was usually completed before the plants showed signs of recovery. These reactions gave strong support to the belief that protective substances were

[1] Received for publication April 26, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 213.

involved in this phenomenon. For convenience, therefore, and because of some similarity to immunological reactions in animals, the terms employed in animal immunology are used in this paper. The term "acquired immunity," as used here, denotes an increase in resistance acquired as a result of some specific reaction between the plant and the virus. This acquired immunity consists of an acquired tolerance of the virus harbored by the plants after recovery and also a resistance to injury from reinoculation. The acquired immunity is referred to as "active" in plants that initiate the reaction leading to recovery and in plants propagated vegetatively from such plants. Plants that have acquired immunity by being grafted with recovered plants are designated as "passively immunized."

This paper reports further on the studies of recovery from curly top, especially that phase of the investigations dealing with the transfer of the acquired immunity from tobacco to tomato.

EXPERIMENTAL RESULTS

ACTIVELY ACQUIRED IMMUNITY IN TOMATO PLANTS

The writer has had few opportunities to look for recovery from curly top in commercial plantings of tomatoes in curly top areas, and only limited information has been obtained regarding the frequency of recovery in plants of tomato varieties under natural field conditions. Over a period of 3 years (1939, 1940, and 1941) about 800 curly top infected plants of commercial varieties were under observation in experimental plots, and only 1 plant recovered. In 1939, at Riverside, Calif., a plant of the Riverside variety recovered from a severe stage of disease by producing basal shoots that showed mild curly top symptoms. This plant had been inoculated earlier by means of leafhoppers (*Eutettix tenellus* (Bak.)) from a mixed colony; i. e., leafhoppers from each of several colonies carrying different virus strains had been grouped on a single large beet plant in order to build up a supply for field inoculations. For inoculation, 20 leafhoppers from this composite colony were confined for 7 days to a terminal portion of a branch of each tomato plant by means of a sleevelike celluloid cage. Cuttings were taken from the recovered Riverside plant and grown in the greenhouse for further study. For identification purposes, the vegetative progeny of this plant was designated Clone 2 and, since the original plant recovered and developed a condition of acquired immunity itself, plants of this clone are considered to have had an actively acquired immunity.

When grown in the greenhouse, cuttings of Clone 2 developed into vigorous plants on which it was sometimes impossible to detect curly top symptoms. Cuttings from Clone 2 were slower in forming roots than were healthy cuttings, and, in the early stages, both root and top growths were somewhat retarded. At later stages, growth of potted plants of Clone 2 very nearly equaled that of healthy plants. At times, slight curly top symptoms were discernible but they were never very conspicuous.

Transfers of virus from plants of Clone 2 to healthy plants by means of the leafhopper vector demonstrated that the plants harbored virus that was virulent on tomato. Figure 1 illustrates typical reactions of

plants used in such a test for virulence. Shown in this figure are (*A*) a vigorous, almost symptomless plant of the actively immunized clone; (*B*) a healthy plant of the same variety inoculated with virus directly from the immunized plant shown, the transfer being made by leafhoppers; and (*C*) a plant of this same variety after inoculation with a known virulent strain of curly top virus. Both inoculated plants eventually died from the effects of curly top; these reactions were

FIGURE 1.—Demonstration of high virulence of virus harbored by plants of actively immunized tomato, Clone 2: *A*, Plant of actively immunized Clone 2; *B*, nonimmunized plant inoculated, by means of beet leafhoppers, with virus from plant *A*; *C*, nonimmunized plant inoculated, by means of beet leafhoppers, with another virus strain known to be virulent on tomato. Both inoculated plants died; this result proved that the virus harbored by Clone 2 was as virulent on tomato as other known virulent strains of the curly top virus.

proof that the virus in the immunized plant was as virulent on tomato as was the other known virus strain.

Clone 2, just described, which had spontaneously recovered from some unknown strain or strains of curly top virus, was tested at different times to two virus strains whose virulence on tomato had been shown. Typical results of such tests are shown in figure 2. Plants *A* and *B* are from immunized Clone 2, and plant *C* is a nonimmunized control. Nonviruliferous leafhoppers were caged on plant *A*, whereas viruliferous leafhoppers carrying a virulent virus strain were caged on plants *B* and *C*. Plant *A*, as expected, was unaffected by exposure to nonviruliferous leafhoppers. A similar cutting from

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the immunized clone showed no reaction to inoculation by means of viruliferous leafhoppers, but the similarly inoculated nonimmunized control plant developed severe symptoms. Under the conditions of these tests, the plants of Clone 2 were highly resistant to or almost completely protected against further injury from at least two virulent strains of curly top virus.

These studies revealed that recovery, although not common, does sometimes occur in cultivated varieties of tomato and that the reactions involved are apparently identical with those previously observed in the studies of recovery of Turkish tobacco from curly top.

FIGURE 2.—Effects of reinoculating plants of actively immunized Clone 2 with another strain of curly top virus known to be virulent. *A*, Plant of immunized Clone 2 exposed to nonviruliferous beet leafhoppers. *B*, Plant of immunized Clone 2 exposed to beet leafhoppers carrying a known virulent strain of virus. *C*, Nonimmunized control plant after inoculation similar to that of plant *B*; plant *C* continued to decline and eventually died from the effects of curly top.

PASSIVE IMMUNIZATION OF TOMATO BY MEANS OF TOBACCO PLANTS

METHODS

In the first tests made to determine whether the acquired immunity of tobacco could be transferred to tomato plants by grafting, healthy tomato scions were grafted laterally on recovered tobacco plants. Such tomato scions did not develop conspicuous curly top symptoms.

This may have been due in part to the fact that the tomato scions were not in a dominant growth position and thus developed too slowly for normal expression of symptoms. After from 4 to 6 weeks, cuttings were taken from the tomato scions and grown in the greenhouse for further study. Plants grown from such cuttings, or by successive propagations from them, developed almost normally, although they grew somewhat slower than healthy plants and usually showed mild curly top symptoms, indicating the transfer of both virus and protective substances from the tobacco plants to the tomato scions.

In later tests, scions from recovered tobacco plants carrying known individual virus strains were grafted to healthy tomato plants. When this method was followed, some of the tomato plants developed mild symptoms from the beginning, while others developed symptoms typical of the early stages of curly top, sometimes resulting in a severe necrosis on upper portions of the terminal shoot. If the growing point was not killed, new growth developing from the original terminal showed progressively less severe symptoms and, in either case, axillary shoots, sometimes almost free of symptoms, arose from below. On the other hand, tomato plants, infected either by direct leafhopper inoculation or by grafting with tomato plants that had been infected by leafhopper inoculation, developed severe curly top and made no recovery.

FIGURE 3.—Severe terminal symptoms on tomato plant grafted with a scion from a recovered Turkish tobacco plant carrying curly top virus, strain 8. The main terminal developed severe symptoms and stopped growth, but subsequently almost normal axillary shoots were produced. Photographed 67 days after the graft was made.

FIGURE 4.—Influence of protective substances on infection of tomato plants with curly top virus, strain 3: *A*, Plant infected by grafting with a tobacco plant that had recovered following inoculation with virus strain 3; *B*, plant infected with the same virus strain by means of beet leafhopper inoculation. Photographed after 64 days.

Figure 3 shows a tomato plant 67 days after it was grafted with a scion from a recovered Turkish tobacco plant carrying curly top virus, strain 8, known to be virulent to tomato. The scion was taken from the terminal of the recovered tobacco plant and was approximately 5 inches in length when the graft was made. The original terminal shoot of the tomato plant was killed, but axillary shoots that were normal in aspect developed from several points along the main stem. Plant *A* of figure 4 was grafted with a scion from a recovered tobacco plant carrying curly top virus, strain 3. Plants of this group did not develop severe symptoms of curly top. For some time the upper parts showed marked leaf rolling, slight yellowing, and some retardation of growth; but, as axillary shoots developed and growth of the original terminal proceeded, the symptoms became less conspicuous. Plant *B* of figure 4 shows the results of infection of a comparable nonimmunized plant with virus strain 3 when the inoculation was made by means of leafhoppers. This photograph was made on the sixty-fourth day after grafting and inoculation of the respective plants. At later stages, plant *B* was dead whereas plant *A* continued growth and was propagated for other tests.

Back Grafts from Passively Immunized Tomato to Healthy Tobacco Plants

The tomato plants that were infected by grafting with recovered tobacco could be propagated vegetatively, but cuttings taken from tomato plants infected by leafhopper inoculation always died. When scions from the passively immunized tomato plants were grafted back to healthy tobacco plants, the tobacco developed only mild symptoms, closely resembling those obtained when grafts were made directly from immunized tobacco to healthy tobacco. On the other hand, healthy tobacco plants grafted with scions from diseased, nonimmunized tomato plants developed severe curly top. Figure 5 shows the striking difference in the reactions of Turkish tobacco plants after grafting with scions from the two sources. The scion used on plant *A* came from a diseased, nonimmunized tomato plant. The scion used on plant *B* came from an immunized plant grown from a cutting from a tomato plant previously infected and immunized by grafting with a recovered tobacco plant. Both plants furnishing scions were in a field inoculation test and had received like inoculations. The reactions of the plants shown in figure 5 are representative of the two groups of plants described in table 1, in which the maximum degree of infection is indicated for each plant. These results, which are typical of those repeatedly obtained, show clearly that the tomato plants that had been infected by grafting with recovered tobacco plants could, in turn, confer protection on other healthy tobacco plants, a situation most simply explained by postulating transfer of a protective principle.

If these reactions are to be interpreted as a type of passive immunization, it must be demonstrated that, in these transfers of virus, the virus itself has not been changed in the direction of attenuation. In the course of these experiments, the virus from the immunized tomato plants was tested by using it to inoculate healthy tomato plants with the beet leafhopper as the vector. In all cases of positive

transfer of virus, the test plants developed severe curly top. Abundant evidence was obtained by tests of this kind that the virus in the mildly diseased, immunized plants produced severe curly top on healthy plants. This evidence proves that recovery of plants and transfer of acquired immunity by grafting cannot be attributed to lessened virulence of the virus. Of course the hypothesis could be

FIGURE 5.—Effects of grafting Turkish tobacco plants with scions from nonimmunized and immunized tomato plants: *A*, Tomato scion from a nonimmunized, leafhopper-inoculated field-grown plant; *B*, tomato scion from a passively immunized plant which had also received a controlled field inoculation. Both field plants furnishing scions had become accidentally infected with tobacco mosaic, but this was not a factor in the reaction of the plants shown.

advanced that the virus in the immunized plants exists in an attenuated state but on passage through the leafhopper an increase in virulence takes place. No parallel or analogous instance of such an effect on the virus is known for curly top. The work of Giddings (*6*), involving thousands of inoculations, has given no indication that curly top strains of low virulence are changed in any way on passage through the leafhopper.

TABLE 1.—*Reactions of healthy Turkish tobacco plants on which were grafted scions from immunized and nonimmunized tomato plants*

[Greenhouse tests]

Source of scion	Reaction [1] of tobacco stock plant—										Scions alive 40 days after grafts were made
	1	2	3	4	5	6	7	8	9	10	
											Number
Immunized tomato [2]	+	+	[3] 0	+	+	+	+	+	+	+	10
Nonimmunized tomato [4]	+++	++++	[5] 0	+++	++	++++	++++	++++	[5] 0	[5] 0	[6] 1

[1] Indicated as follows: 0=no reaction; +=mild; ++=moderately severe; +++=severe; ++++=extremely severe.
[2] Plant grown from cutting from tomato plant immunized by grafting with an immunized tobacco plant.
[3] Scion lived but made no growth; stock plant not infected, indicating absence of vascular connection between scion and stock.
[4] Tomato plant infected directly by leafhopper inoculation.
[5] Scion died early; apparently no vascular connections formed.
[6] 1 scion remained alive but made no growth; stock plant became diseased.

GREENHOUSE REINOCULATION TESTS ON PASSIVELY IMMUNIZED TOMATO PLANTS

Several different tomato clones that originated from tomato plants passively immunized by grafting with recovered tobacco plants were

FIGURE 6.—Effects of reinoculation with a virulent strain of curly top virus upon passively immunized tomato plants: *A*, Control plant, showing extremely severe injury; *B* to *D*, plants from passively immunized clones, showing no conspicuous injury from inoculation.

grown in the greenhouse and tested for resistance to reinfection. In the early tests, plants of these immunized tomato clones were reinoculated on several occasions with unknown mixtures of curly top virus

strains. In other tests, two known strains were used individually for reinoculation. Although the virus strains used were virulent on tomato and produced severe symptoms on nonimmunized healthy control plants, the immunized plants showed no noticeable reactions to these reinoculations. The effects of inoculation upon a healthy control plant and three immunized plants, all grown as cuttings, are shown in figure 6. It can be seen that the healthy control was severely injured by the inoculation, but the immunized plants were unaffected. Similar reinoculation tests were made on a sufficient number of plants to justify the conclusion that the tomato plants, after being passively immunized from tobacco, were provided with a high degree of protection against two known strains and certain other unknown strains of curly top virus. Additional data concerning reinoculation of immunized tomato plants are presented later in this paper. In the experiments just described, records were incomplete in regard to the virus strains present in some of the immunized clones. These preliminary data have been presented, however, because they demonstrated passive immunization and because these clones were used in subsequent field tests.

FIELD TESTS OF IMMUNIZED TOMATO PLANTS

Natural Exposure

In 1940 field plantings were made of some of the immunized tomato clones near Twin Falls, Idaho. Actively immunized Clone 2 and four clones of passively immunized tomatoes were included, as well as healthy, nonimmunized plants for controls. One of the passively immunized tomato clones was of the Stone variety and the others were of the Riverside variety. The cuttings were rooted at Riverside, Calif., and shipped to Twin Falls, where they were potted and grown in the greenhouse for about 3 weeks before being transplanted to the field.[3] The controls consisted of cuttings from healthy, nonimmunized Riverside plants; as a further check on the degree of exposure to curly top, seedling plants of both the Riverside and the John Baer variety were included in the field planting.

The plants were set in the field on June 12 in a plot bordered on both sides by sugar-beet plantings. This Idaho district experienced an extremely heavy infestation of beet leafhoppers, and the incidence and severity of curly top in the nonimmunized tomato plants and in the susceptible sugar-beet varieties nearby gave proof that the tomato plants had had a severe curly top exposure. During the early part of the season, before curly top began to appear, the healthy control plants grew much more vigorously than the immunized plants. However, as the season advanced and the disease spread through the controls, the immunized plants continued to grow and increase in size, whereas by September 1 the control plants, numbering 224, were all severely diseased and a large percentage of them had died. Curly top was so severe and developed so rapidly that no edible fruits were produced on the controls. From September 1 until the plants were killed by frost, there was a sharp contrast in the appearance of the immunized lots and the nonimmunized controls. With the exception of the plants of one clone, a high percentage of the immunized plants were in good

[3] The Idaho field plantings were supervised by Albert M. Murphy, assistant pathologist, Division of Sugar Plant Investigations.

View of a portion of experimental planting of immunized and nonimmunized tomato plants. Center foreground, left to right: Row 1, nonimmunized control grown from cuttings; row 2, plants of actively immunized Clone 2; row 3, plants of passively immunized Clone 3; row 4, plants of passively immunized Clone 4, some showing injury from reinfection with curly top virus. Photographed September 11, 1940, Twin Falls, Idaho.

condition, showing almost normal color, growing fairly vigorously, and producing a light to medium crop of fruit of good quality. Although some fruit ripened on the immunized plants on about the normal date, ripening in general was somewhat delayed and the plants had many green tomatoes when killed by frost. The fruits that matured on the immunized plants were graded as small to medium, and the yield, although good on some plants, was generally lower than would have been expected on plants not affected by curly top.

Plate 1 shows a part of the 1940 Idaho plot as it appeared on September 11. The rows of nonimmunized control plants, consisting of severely yellowed or dead plants, are easily located. In contrast, the immunized clones (replicated throughout the plot as single rows of 10 plants each) for the most part show no injury. In the foreground, as marked by the numbered stakes, are shown (1) dead or badly diseased plants grown from cuttings of nonimmunized healthy plants, (2) highly resistant plants of Clone 2 (actively immunized), and (3) highly resistant plants of Clone 3 (passively immunized). Adjacent to and on the right of Clone 3 can be seen a portion of a row of another passively immunized clone. The yellowed plants of this latter group show injury from reinoculation.

TABLE 2.—*Reactions of immunized tomato clones and of healthy, nonimmunized controls to curly top exposure in field tests in Idaho*

[Record made October 2, 1940]

Seedling lot or clone No.	Variety and immunization	Virus strains used in immunizing	Plants tested	Plants diseased	Plants showing indicated degree of infection [1]			Plants surviving
					Severe	Moderately severe	Mild	
			Number	*Number*	*Number*	*Number*	*Number*	*Percent*
1	Cuttings, Riverside variety, nonimmunized controls.	None	76	76	76	0	0	26.4
2	Cuttings, Riverside variety, actively immunized.	Mixture [2]	57	[3] 57	7	0	50	100.0
3	Cuttings, Stone variety, passively immunized.	do [4]	39	[3] 39	0	6	30	[5] 92.3
4	Cuttings, Riverside variety, passively immunized.	Strain 9 [6]	61	[3] 61	[7] 6	13	34	86.9
5	Cuttings, Riverside variety, passively immunized.	Mixture [4]	45	[3] 45	0	3	42	100.0
6	Cuttings, Riverside variety, passively immunized.	Strain 9 [6]	28	[3] 28	3	19	6	100.0
7	Seedlings, Riverside variety, nonimmunized controls.	None	74	74	74	0	0	0
8	Seedlings, John Baer variety, nonimmunized controls.	do	74	74	74	0	0	0

[1] All plants in immunized groups not showing symptoms were included as mildly diseased.
[2] Original plant recovered after inoculation with mixture of strains.
[3] All immunized plants carried virus and were therefore considered to be diseased regardless of symptom expression.
[4] Immunization came from tobacco plant that had been inoculated by leafhoppers from stock colony presumably consisting of mixed strains.
[5] 3 plants died from ground squirrel injury; from standpoint of curly top, this group also had 100-percent survival.
[6] Immunization came from tobacco plant that had been inoculated by leafhoppers carrying a single known strain (strain 9) of virus.
[7] This number probably low; 8 dead plants were not included because of uncertainty of cause of death.

The different tomato groups used in this test are listed in table 2, with data on reaction to the curly top exposure and plant survival on October 2. Plants of immunized Clone 6 were known to be low in

vigor. Growth was poor, and the plants had shown marked curly top symptoms when grown in the greenhouse without further exposure to infection. Therefore, the poor performance of this clone, as shown in table 2, was very probably not due to reinfection, but rather to the fact that these plants had a lower degree of specific protection against the virus already in them. It is of interest, however, to note that 100 percent of the plants of this clone survived, even though more than three-fourths of them showed symptoms ranging from moderately severe to severe.

The data on survival of plants, as shown in table 2, contribute significant information. From the standpoint of mortality from curly top, 4 of the 5 immunized lots gave 100-percent survival. In Clone 3, shown as 92.3-percent survival, the loss of 3 plants from causes other than curly top was responsible for this group not being recorded as 100 percent. In Clone 4, a total of 8 plants died, but it was not possible to determine definitely whether all of these died from curly top. Even if all of the 8 plants died from curly top, this would make a total loss of only 8 out of 230 immunized plants in the test as a whole. On the other hand, in the controls all of the 148 healthy seedlings were dead on October 2, and only 20 out of a total of 76 plants grown from nonimmunized cuttings were surviving on that date. Of the 20 surviving plants in this latter group, all were severely diseased, and many were surviving only because of the fact that frequent rains and much cloudy, cool weather in September had enabled them to persist.

Some of the plants of the immunized groups, particularly in Clones 2, 3, and 4, developed curly top symptoms decidedly more severe than those shown by the majority of the plants in these groups. The most reasonable explanation of this seemed to be that for the most part the immunized plants were protected to a high degree against injury from the virus strains carried by most of the beet leafhoppers but that some of the vectors transmitted other strains against which the protection by immunizations was not equally effective. It was also evident that some of the immunized lines had a higher degree of protection than others. Other data, presented in a later section, support this explanation of the results obtained.

Controlled Inoculations

At Riverside, Calif., in 1940, field plantings were made of the immunized clones included in the Idaho tests, with the exception of Clone 6. The stock plants of this clone were of such a low degree of vigor that no plants of this line were available. Cuttings from healthy plants and healthy seedlings of the Riverside variety were included as controls. Since natural infestation in this area is usually very light, controlled inoculations can be made in the field, and the desired noninoculated plants, for the most part, remain free of infection throughout the season. These conditions permitted observations on both reinoculated and untreated plants of the immunized groups and on inoculated and noninoculated controls.

Well-established potted plants were transplanted to the field on May 29, and inoculations were made on July 5. Leafhoppers from each of several colonies, carrying different virus strains, were grouped, and 20 leafhoppers from the mixed colony were caged on each plant to be inoculated. All of the curly top strains used were known to be virulent on healthy tomatoes.

The inoculation tests at Riverside gave conclusive evidence that, under the conditions of this experiment, the immunized clones were injured by reinoculation when many virulent curly top virus strains were used. Certainly, one or more of the virus strains used for inoculation were capable of reinfecting the plants and producing severe injury. Another obvious fact was that some of the immunized clones responded differently to this reinoculation. This fact becomes evident upon examination of the data presented in table 3, prepared from records taken on September 16. The tests under natural exposure in Idaho also gave a suggestion of such differences and, of the three passively immunized clones tested in both localities, Clone 4 in both instances showed the most injury from reinfection. A possible explanation for a lower degree of protection in this clone is that the immunization in these plants originally came from a tobacco plant carrying a single strain of virus, whereas in Clones 3 and 5 the immunization came from tobacco plants originally inoculated with a mixture of strains, all of them different from that in Clone 4. As will be brought out later, the degree of protection may vary between immunized clones harboring different strains of the virus.

TABLE 3.—*Reaction of immunized tomato clones and healthy nonimmunized controls to curly top in field inoculation tests at Riverside, Calif.*

[Record made September 16, 1940]

Seedling lot or clone No.	Variety and immunization	Virus strains used in immunizing	Field treatment	Plants tested	Plants showing indicated degree of infection			Plants surviving
					Severe	Moderately severe	Mild	
				Number	*Number*	*Number*	*Number*	*Percent*
1	Cuttings, Riverside variety, nonimmunized controls.	None	Inoculated	20	20	0	0	65.0
			Not inoculated	16	0	0	0	100.0
2	Cuttings, Riverside variety, Clone 2, actively immunized.	Mixture [1]	Inoculated	20	7	10	3	75.0
			Not inoculated	20	1	0	19	100.0
3	Cuttings, Stone variety, passively immunized,	do [1]	Inoculated	19	2	2	15	100.0
			Not inoculated	19	0	1	18	100.0
4	Cuttings, Riverside variety, passively immunized.	Single strain [2]	Inoculated	19	15	2	2	36.8
			Not inoculated	19	0	8	11	100.0
5	Cuttings, Riverside variety, passively immunized.	Mixture [1]	Inoculated	30	11	14	5	93.3
			Not inoculated	30	0	0	30	100.0
7	Seedlings, Riverside variety, nonimmunized controls.	None	Inoculated	10	10	0	0	0
			Not inoculated	8	0	0	0	100.0

[1] Undetermined mixed strains of virus, used in original inoculations of the tomato or tobacco plants in which the immunization was first initiated.

[2] A single known strain (strain 9) of virus, used in inoculation of the tobacco plant in which the immunization originated prior to its passive transfer to tomato.

ROLE OF VIRUS STRAINS IN ACQUIRED IMMUNITY OF TOMATO CLONES

REACTION OF PLANTS OF ACTIVELY IMMUNIZED CLONE 2 TO DIFFERENT VIRUS STRAINS

Inoculation tests of immunized tomato plants under controlled conditions in the greenhouse had proved that the plants were highly resistant to injury from infection with at least two strains of curly top virus that were virulent on nonimmunized plants. The reaction of

some of the immunized plants under natural exposure in the field at Twin Falls was interpreted to mean that protection from one strain

FIGURE 7.—Representative tomato plants from reinoculation test in which plants of actively immunized Clone 2 were inoculated with different strains of the curly top virus. *A*, Plant inoculated with strain 8; unaffected. *B*, Plant inoculated with strain 66; mild symptoms developed on terminal portions but plant continued growth without being stunted. *C*, Plant inoculated with strain 58; conspicuous symptoms developed; terminal growth weak and chlorotic. Photographed 76 days after inoculation. Of the nonimmunized control plants inoculated at the same time with these three strains of virus, none survived.

of the virus did not give protection against all strains. Artificial-inoculation tests of field-grown plants at Riverside gave conclusive proof that acquired immunity against one strain of the virus was not

effective against all strains, and this phenomenon presented a new phase of the problem for investigation. Although this part of the study is still incomplete, some of the results obtained are reported here.

The previous tests with Clone 2 had demonstrated that the acquired immunity in this clone was effective against two virus strains. This plant material was chosen for further tests against a number of virus strains. These tests demonstrated that virus strains play a significant role in acquired immunity from curly top in tomato. By making tests with a number of strains of curly top virus, it was shown that, depending upon the strain used, the protection existing in Clone 2 ranged from complete to slight. The plants shown in figure 7 are representative of three groups of Clone 2, 76 days after inoculation with three strains of virus respectively. Plant *A*, inoculated with strain 8, was unaffected; plant *B*, inoculated with strain 66,[4] gave a mild reaction; and plant *C*, after reinfection with strain 58, developed severe symptoms.

The three virus strains used in this test are virulent on healthy tomato plants. In the test just described, healthy plants were inoculated in each case and all of them died from curly top before the plants in figure 7 were photographed. Although the plants of this immunized clone reacted quite severely to reinoculation with virus strain 58, the development of disease was much slower than in healthy plants infected with this strain, and usually the reinfected immunized plants survived for long periods. In fact, some of them made a feeble recovery and could be continued by cuttings, but the plants propagated from them were of very low vigor. However, it was evident that the immunized plants were more resistant to virus strain 58 than the nonimmunized plants.

TABLE 4.—*Reaction of plants of actively immunized Clone 2 and of healthy nonimmunized control plants to inoculation with 9 different virus strains*

[The number of plants of each clone tested against each virus strain ranged from 4 to 15]

Tomato clones	Reaction [1] when inoculated with virus strain—								
	1	3	5	6	8	9	58	60	66
Clone 2, immunized [2]	0	0	+++	+++	0	+++	+++	0	+
Clone 1, nonimmunized [3]	++++	++++	++++	++++	++++	++++	++++	++++	++++

[1] Indicated as follows: 0 = no reaction; + = mild; +++ = moderately severe to severe (some plants died after long periods, others made weak recovery); ++++ = extremely severe, causing death of all plants.
[2] Virus strains involved in the initial active immunization were not identified.
[3] Healthy controls.

The reaction of plants of actively immunized Clone 2 to reinoculation with nine different strains of curly top virus has been determined. Table 4 summarizes these data and shows, in comparison, the reaction of healthy nonimmunized tomato plants to the same nine strains. Four strains failed to produce any noticeable effect on Clone 2; the protection, under the conditions of these tests, was complete against those strains. However, the other five strains infected the immunized plants, and four of them caused marked injury. Although

[4] Strain numbers above 10 are tentative numbers assigned by N. J. Giddings for identification purposes.

this clone was quite severely affected when reinoculated with certain virus strains, the plants were very slow in showing the effects of reinfection; and some of them survived and sometimes made a partial recovery. On the other hand, the healthy controls developed curly top rapidly and all of them died as a result of infection.

Increased manifestation of symptoms following reinoculation of immunized plants seems to be sufficient proof that the virus strain used for reinoculation became established in the plants. Nonviruliferous leafhoppers were fed on some of these plants to acquire virus and then were transferred to differential host plants whereby it could be determined if the second virus strain was present. In this manner, for example, it was shown that virus strain 58 was present in some of the Clone 2 plants that had been inoculated with this strain.

Reaction to Different Virus Strains of Clones Passively Immunized Against Single Known Virus Strains

In order to make a further study of the relation of virus strains to acquired immunity from curly top, tomato plants were passively immunized against 12 individual strains of virus. To accomplish this immunization, Turkish tobacco plants were inoculated separately, by means of leafhoppers, with known curly top virus strains. Forty days after inoculation some of the tobacco plants had made a good recovery, others were in various stages of recovery, and some still showed no signs of recovery. At that time, healthy tobacco plants were grafted with scions from the inoculated tobacco plants, each carrying a different virus strain. The graft-infected plants developed mild symptoms in all instances, proving that the reactions leading to recovery had taken place in the leafhopper-inoculated plants even though some of them had not yet begun recovery. All of these plants produced new growth later, and there appeared to be no consistent differences in vigor and symptom expression between plants infected with different strains of the virus. A similar uniformity was displayed by the tobacco plants that were immunized by grafting with the leafhopper-inoculated plants.

After the acquired immunity against the individual strains of virus was established in the series of tobacco plants, scions from them were grafted to healthy Riverside tomato plants, to serve as the foundation plants from which to establish 12 tomato clones, each specifically immunized against, as well as carrying, a different virus strain. Later, similar graft transfers were made from immunized tobacco to plants of the tomato varieties John Baer, First Early, and Break o' Day. In some of these experiments, comparable tomato plants were grafted separately with scions from an immunized tobacco plant and with scions from a tomato plant that had been immunized previously from this same tobacco plant. This was done to determine whether the degree of protection conferred on tomatoes was affected by the source of protective substances. From the data so far obtained, there was no good evidence that such was the case. For instance, with a given strain of virus, tomato plants usually reacted about the same when infected by grafting directly with an immunized tobacco plant as when the infection came from a tomato plant which had previously been passively immunized from that same tobacco plant. Some differences in reaction appeared but they were not consistent.

It has been mentioned already (p. 202) that Turkish tobacco plants immunized against the individual virus strains were quite uniform in appearance and vigor. Thus it appeared that tobacco plants recovered to approximately the same degree regardless of which strain of virus was involved. However, when tomato plants were passively

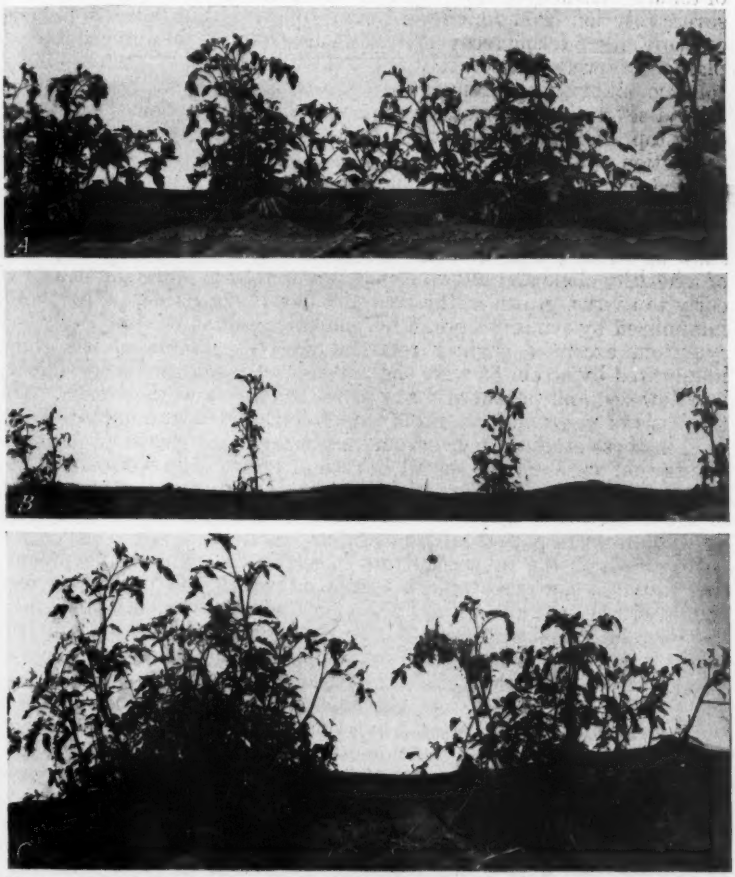

FIGURE 8.—Reduction in vigor of immunized tomato clones, and variation between clones immunized by different strains of curly top virus: *A*, Individuals of clone immunized by virus strain 3; *B*, individuals of clone immunized by virus strain 55; *C*, healthy clonal plants for comparison. Cuttings were made at same time. Photographed 2 months after transplanting to field. Healthy control plants grown in 3-foot spacings, others in 2-foot spacings.

immunized against different strains of curly top virus, the results indicated that the degree of immunization attained by the plants varied widely with different virus strains. This was shown by variations in both the initial reaction of the tomato plants after they were grafted with immunized tobacco scions and in the vigor of the clonal propagations from these tomato plants. Tomato plants grafted with

immunized tobacco scions carrying certain virus strains developed severe terminal symptoms and then produced mildly diseased axillary shoots (fig. 3). When certain other virus strains were used, the graft-infected tomato plants showed no severe initial shock such as that previously described (fig. 4). With still other virus strains, infection of tomato plants by graft transfer from immunized tobacco produced a severe reaction from which the tomato plants either failed to recover or made only a feeble recovery. Cuttings from the immunized tomato plants that were not severely affected after grafting with immunized tobacco, and cuttings from tomato plants that made a strong recovery after a severe initial reaction, grew vigorously and showed very mild curly top. Clones of the tomato plants that acquired a low degree of protection against some of the virus strains were low in vigor and showed marked injury from curly top.

Variations in vigor and symptom expression among the various immunized tomato clones, each carrying a different strain of the virus, show up strikingly prior to any reinoculation. Figure 8 shows plants of a healthy clone and of two clones immunized by different strains of curly top virus, grown in the field and not reinoculated. The plants immunized by strain 3 were of normal color and showed no curly top symptom except a slightly retarded growth. Plants of the clone immunized by strain 55 were slightly chlorotic and grew very slowly, but flowered and produced a few small fruits late in the season. On the basis of vigor and symptom expression, the 12 immunized clones used in these studies ranged from very sickly, slow-growing plants to plants that were normal except in rate of growth. In some instances plants of a particular clone, although small to medium, showed none of the usual curly top symptoms. Plants within a given clone were quite uniform in growth and general appearance.

Inasmuch as the tobacco plants in which the immunizing process was initiated and the tomato plants on which the protection was conferred were not all of single genotypes, some of the variability may have come from this source. However, because of the very uniform reaction in tobacco and general concordance among tests with various tomato stocks, effects of variability of the host plants are probably not the significant factor. From the evidence so far obtained, it seems that the immunization acquired by tomato is chiefly influenced by the virus strain itself and the reactions that it sets up.

Although field tests under natural exposure yield much worth-while information, controlled cross-inoculation tests are necessary to determine the relation of virus strains to acquired immunity from curly top in tomatoes. This represents a protracted study, which is still in progress; but some of the preliminary data can be presented.

Several tomato clones, each passively immunized by grafting with tobacco plants that had recovered after infection with single strains of the virus, were subjected to reinfection tests with various strains of the virus. The results of these tests, so far as they have gone, are summarized in table 5. In most instances, from 10 to 20 plants of each clone were inoculated with each virus strain used. The data show that, under the conditions of these tests, plants immunized by one strain may be protected against many strains but that the protection given by one strain of virus does not correspond with that given by some other strain. Of especial interest are the reactions of

the clones immunized by different virus strains to reinoculation with strains 9 and 75. The intensity of curly top reactions on some of the immunized clones in these tests might, at first glance, indicate a greater virulence in these two strains; nevertheless all the other virus strains likewise had a lethal effect on the controls. It is to be noted that the tomato plants immunized by strain 55 were completely protected against both 9 and 75, but that strains 5 and 6 both injured to some degree plants immunized by strain 55. In view of the fact that other data presented in this paper have established good evidence that protective substances are involved in recovery and acquired immunity from curly top, the strain specificity just described is interpreted as further evidence that the protective substances are of an immunologic nature. The failure of related virus strains to provide cross-immunity has been demonstrated on several occasions in the field of animal pathology (*11*, *16*).

TABLE 5.—*Reactions of immunized tomato clones carrying single virus strains to inoculation with various strains of curly top virus*

[From 10 to 20 plants of each clone were tested against each virus strain]

Plants immunized against virus strain No.	Reaction [1] when inoculated with strain—									
	3	5	6	8	9	53	55	56	60	75
3	0	++	++	0	+++	0		0		0
8	0			0	+++	0	0			0
53	0	0	0	0	++	0	0		0	++
55	0	+	+	0	0	0	0			0
56	0			0	++	0	0	0	0	+++
Controls (nonimmunized)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

[1] Indicated as follows: 0=no reaction; +=mild; ++=moderately severe; +++=severe; ++++=extremely severe, causing death of all plants.

DISCUSSION

In contrast to other virus diseases of plants that have been studied in relation to recovery and acquired immunity, curly top virus is restricted to the phloem and does not normally invade parenchyma tissues (*2*). Inoculation, except with the vector, gives a very low percentage of infection, apparently because the virus must be introduced directly into the phloem cells. Bennett (*3*) has shown that the virus may invade healthy mature leaves of sugar beet and multiply in them. There are, however, no primary localized symptoms at the site of inoculation such as commonly occur with the ring spot group of viruses. Instead, the symptoms of curly top appear after systemic invasion of tissues that have not passed a certain stage of maturity. Because the tissue relationship of curly top virus and the type of symptoms and manner in which they develop differ so distinctly from other viruses in which recovery has been studied, it was of particular interest to discover this recovery reaction in curly top affected plants.

Several workers have expressed the opinion that, in view of the lack of information on the nature of the reactions involved in recovery of plants from virus diseases and the accompanying protection, it is preferable to refer to this condition of the recovered plants as "acquired tolerance." Valleau (*19*, *20*) has presented strong arguments

against the use of the terms "recovery" and "acquired immunity" in connection with the reactions of tobacco plants affected with ring spot. Regarding the mechanism involved in these phenomena, Price (*14*) and Valleau (*19*) seemed to agree that patterned leaves are produced as long as the growing point has not been invaded and that, once the embryonic cells become invaded, patternless leaves are formed. Price (*14*) offered the hypothesis that ring spot virus reaches its maximum concentration in and exerts its maximum effect upon only those cells that are nearly mature at the time they are infected; and, further, invasion of embryonic cells is not accompanied by maximum increase in virus nor by severe injury to the cells. Thus embryonic cells, once invaded, become adapted to the presence of the virus and establish an equilibrium with it. Price recognized that, regardless of the correctness of this hypothesis, it still did not reveal the fundamental nature of the mechanism involved; he expressed the opinion that these reactions are sufficiently similar to many known immune reactions in the field of animal viruses to be accepted as examples of acquired immunity.

Valleau (*19*, *20*) contended that, in the case of ring spot and certain similar viruses, the reactions involved in recovery are in no way immunological. He agreed with Price that the acquired tolerance is cellular, but suggested that embryonic cells become tolerant of the virus because of the presence of less virus-precursor materials or because of a less rapid change of such materials into virus. Other workers (*1*, *8*, *10*, *18*) have suggested a somewhat similar explanation for instances of cross-protection, wherein a second virus fails to produce symptoms when inoculated into a plant previously invaded by a closely related virus. It seems logical to assume that certain cell constituents are essential for virus multiplication, and if such is the case the essential materials in a cell invaded by a given virus may become exhausted or at least be used as fast as they are synthesized. Under such conditions it would hardly be expected that a second virus, requiring the same materials as the virus already present, would become established in concentrations sufficient to produce its symptoms when inoculated into cells that are, as stated by Valleau (*19*), "already parasitized to their limit."

Price (*13*) grafted from ring spot recovered plants to healthy plants and found that typical symptoms of the severe stage always developed on the graft-infected plants, the reaction being identical with that on plants infected by the leaf-rubbing method. He stated that, if protective substances were present in the recovered plants, they were not detected.

In the case of curly top, the discovery that usually tobacco plants developed only mild disease symptoms after being grafted with recovered tobacco plants strongly indicated that protective substances were present in recovered plants (*23*). This conclusion was supported by the fact that the recovered plants contained virus that caused severe curly top when transferred by the leafhopper vector to healthy plants. When reinoculation tests had shown that recovered tobacco plants and their vegetative progeny were unaffected by reinoculation with curly top virus and, further, that tobacco plants that were infected by grafting with recovered plants were provided with a similar protection (*23*), it was concluded that in this instance the recovery

reactions were of an immunologic nature. Plants that recovered from a severe stage of curly top and were unaffected by reinoculation were described as having an actively acquired immunity. The failure of a healthy tobacco plant to develop severe curly top when grafted to an immunized plant was attributed to a type of passive immunization. Because the condition of immunity acquired by plants that recovered from curly top could be transferred through grafts to other plants, it has been possible to carry these investigations much further than in other instances of recovery of plants from viruses, such as tobacco ring spot, where recovered plants confer no protection on plants to which they are grafted.

Wallace (*23*) has shown that, under conditions about optimum for rapid curly top development, tobacco plants inoculated by means of leafhoppers required a period of about 20 days before they reached a condition that permitted them to confer maximum protection on healthy plants by passive transfer. On the other hand, if scions taken from leafhopper-inoculated plants on the fifth day following inoculation, prior to any showing of curly top symptoms, are grafted on healthy tobacco plants, severe symptoms are shown by the stock plants. It was therefore concluded that passive transfer of protection from plant to plant by grafting resulted from some reaction or change that occurs in leafhopper-inoculated tobacco plants sometime between the fifth and twentieth day after infection. Graft transfers at intermediate periods gave results that indicated that the reaction had begun within 10 days but was not completed on the fifteenth day after the plants were infected. Each of the time periods mentioned above actually may be longer by about 7 days, since in tobacco phloem connection between scion and stock usually requires 7 days or more. Thus, there is the period from time of grafting to time of phloem union, in which the reaction may continue in the scions before phloem materials can move into the stock plants. At the time the grafts were made, the 5-day infected plants showed no curly top symptoms, but the 20-day infected plants showed severe symptoms. On the basis of other tests with the curly top virus, it is believed that, if there is a difference in virus concentration between plants infected 5 days and plants infected 20 days, the scions from the 20-day infected plants contain the higher concentration. At any rate, the virus concentration in the respective scions is not believed to have been responsible for the results obtained in these tests. Plants infected by grafting, regardless of the source of scions, certainly must receive more virus than can be introduced by a small number of leafhoppers, yet infection from leafhopper inoculation usually causes severe symptoms. Leafhopper-inoculated tobacco plants and plants grafted with scions from short-time-inoculated plants sometimes develop only mild symptoms when the incubation period is long. In graft infections a delay of a few days after the graft union is formed before virus moves into the stock may permit the completion of the protective reaction before the terminal of the stock is invaded.

The evidence that tobacco plants develop some type of defense against the virus, enabling them to recover, became more striking when it was shown that, by grafting with immunized tobacco plants, it was possible to immunize tomato varieties that rarely recover and acquire immunity actively when inoculated by means of leafhoppers. The effectiveness of this method also demonstrated conclusively that,

whatever the defense mechanism is, it can be transferred through grafts to other plants and can function there in providing protection. After tomato plants have been passively immunized from tobacco plants, this condition can be passed on from these plants to other tomato plants by grafting. If, on the other hand, healthy tomato plants are grafted with diseased nonimmunized tomato plants they become severely affected with curly top and do not recover. Such results cannot be explained on the basis of invasion of embryonic tissues, since the opportunity for invasion of the graft-infected plants is the same in either case. The different response of tomato plants infected from immunized and from nonimmunized scions must, therefore, be caused by a difference in the scions themselves.

That invasion of immature tissues may play some role in acquired immunity from curly top is indicated by some of the results obtained in the studies with tobacco. Wallace (*21*) reported that, when tobacco plants were topped at the time they were grafted with an immunized scion, the symptoms that developed on the first axillary shoots were usually fairly severe for a time. However, such shoots recovered sooner than similar shoots on plants infected by leafhopper inoculation or by grafting with nonimmunized scions. Although this reaction might indicate some relation between stage of tissue differentiation and invasion by the curly top virus, such a relation would be the opposite of that existing in the case of invasion by the ring spot virus; invasion in the earlier stages by curly top causing a severe reaction, whereas a similar invasion by ring spot virus produces symptomless tissues.

Tobacco plants that are not topped after grafting with an immunized scion usually develop mild symptoms from the beginning. Occasionally, however, an untopped plant may develop quite marked symptoms; this is particularly true if the graft-infected plant is closely approaching or has reached the flowering stage. Plants that have shown very mild symptoms for a long time sometimes develop conspicuous symptoms if the plants are topped so as to force new growth from axillary buds. These reactions, and also the increased symptoms on new growth of plants that are topped at the time they are grafted with an immunized scion, are believed to result from an increase in virus concentration through the movement of virus, and possibly of materials used in virus multiplication, into these areas from other parts of the plants. It is known that viruses move rapidly in the phloem toward regions of food utilization or storage. If this rapid movement results from mass flow of the liquid contents of the phloem, as has been suggested by Bennett (*3*), it would be expected that viruses would become highly concentrated in areas receiving large quantities of food materials from other parts of the plant. Bennett and Esau (*4*) found that the concentration of curly top virus increased rapidly in seeds of sugar beet as the seeds developed on curly top affected plants. In consideration of the above points, it would seem that in the plants immunized from curly top the mild symptoms and lessened injury are due to the fact that the virus concentration usually is held at a low level, i. e., at concentrations too low to produce normal injurious effects. The nature of the mechanism that causes this condition is not understood. The studies reported here indicate very strongly that plants recover and acquire this type

of protection from curly top as a result of some reaction between the virus and certain materials in the plants, resulting in the production of specific substances that either have an inhibiting effect on the virus directly or else bring about some change in the plants themselves, enabling them to tolerate the virus without becoming seriously injured.

The role of curly top virus strains in acquired immunity is particularly interesting. Turkish tobacco plants seem to recover to about the same degree regardless of the strain of virus in them. However, when graft transfers are made from tobacco to tomato plants, a high degree of protection is acquired by tomato against some of the virus strains and protection of lower degree is acquired against other strains. The conclusion that this difference exists is based on the observation that immunized tomato clones, carrying different strains of virus, vary widely in vigor and severity of symptoms. There has been some indication that individual plant variation has some influence on the degree of protection acquired by tomato. Slight genetic differences in the tobacco plants that develop active immunity or in the tomato plants that are passively immunized could be responsible for some of the variations in the degree of immunization acquired and maintained. Incomplete studies along this line indicate that if a sufficient number of seedling tomato plants are passively immunized against a virus strain that consistently provides a low degree of protection in tomato, an occasional tomato plant in the group may acquire protection of a much higher order, from which a vigorous clone can be established. However, the present belief is that the virus strain itself and the reactions it sets up in the plant account for most of the observed variation between tomato clones immunized by different strains of curly top virus.

Another interesting reaction is revealed by the discovery that immunized tomato plants carrying a certain virus strain or combinations of strains are unaffected by reinoculation with some strains and are injured quite severely by others. As in other phases of this study, the question arises as to whether these data provide any evidence concerning the nature of recovery and acquired immunity from curly top. In attempting to answer this question, a search for analogous reactions in the field of animal viruses has been made. According to Rivers (*16, p. 215*), there are three distinct types of the virus of foot-and-mouth disease, which, although they cannot be distinguished from one another clinically, do not produce cross-immunization. Rivers points out also that there are two types of the virus of vesicular stomatitis of horses, producing identical clinical pictures, but that animals recovered from one are not immune from the action of the other. Another example can be cited in the case of the influenza virus. There are different types of this virus and, further, there are different strains of a given type. Some of the strains of human influenza A virus have been shown to differ widely in antigenic make-up (*11*). This fact has been demonstrated both by reciprocal cross-neutralization tests and cross-immunity tests. Thus, it seems that a plurality of types exists in certain animal viruses whereby a particular virus may consist of two or more strains that differ in their immunologic reactions. In tomato plants, the failure of immunity acquired against one strain of the curly top virus to be effective against all strains of the

virus is similar in some respects to the animal virus reactions just described.

Putting aside connotations of terminology involved in the word "immune," the objections most commonly expressed to the findings that plants may acquire immunity against a virus disease are based on the fact that the plants do not completely recover from the disease symptoms and that the causative virus is at all times present in the plants. In other words, insistence is made that recovery or acquired immunity be limited to return to a virus-free condition. These objections are based in part on the assumption that in the field of animal immunology no analogous conditions exist and that animals that recover and acquire immunity from a virus make a complete recovery from disease symptoms and become free of the virus. Good evidence has already been obtained that immune animals do not always become virus-free, and some suggest that they may never reach this condition. Rivers (*16*, *17*) cites instances where the viruses of certain diseases have been demonstrated to be present in recovered animals for quite a long period after the symptoms of the disease had disappeared. In fact, it has been suggested that the lasting immunity from many diseases of man and lower animals may actually be dependent upon the persistence of virus (*16*, *17*, *25*, *26*).

There is such a wide difference between plant and animal organisms that it could hardly be expected that they would present identical pictures after acquiring immunity against infectious agents. Higher plants are in a continuous process of growth, particularly when kept in a vegetative state. New tissues are being formed at a rapid rate, perhaps much faster and more generally than in animals, and these tissues very probably produce a continuous supply of materials needed for virus multiplication. The formation of new tissues likewise furnishes susceptible tissues in need of protection. A small cutting from a plant that is immunized against a virus disease increases in size manyfold. Under such conditions, would it not be expected that plants would require a far higher degree of immunization if they are to be protected to the same extent as immunized animals?

The argument is frequently put forth that the wide difference between plant and animal circulatory systems makes it doubtful whether plants can develop a defensive mechanism similar to the antibody formation of animals. In the case of curly top, the virus is restricted to the phloem and thus exists in a uniform medium. If immune reactions occur in the plant virus field, it seems somewhat more probable that they would be found in phloem-restricted viruses. If protective substances are produced in the case of a phloem-restricted virus they would have to exist and operate only in one type of tissue in order to provide a defense against the virus. On the other hand, with viruses that are not restricted to the phloem, the defense mechanism would be required to operate in all of the different tissues invaded by the virus. Under such conditions, it would seem less likely that effective protection would be attained.

If plants that recover from curly top actually contain some type of protective materials, the failure of the leafhoppers to transmit them, assuming mere quantitative considerations are not involved, suggests that these materials are either inactivated in the leafhopper or that they are screened out in some manner so that they do not

reach the salivary glands of the insect. The leafhopper apparently separates the virus from the protective substances or else acquires and transmits only free virus, i. e., virus that has not been acted upon by protective substances.

Recovery and acquired immunity from curly top cannot be explained satisfactorily by curtailment or exhaustion of essential materials for virus reproduction or by invasion of embryonic cells. In the first place, in sugar beets, which do not recover and acquire immunity from curly top, ordinary cross-protection between virus strains does not occur. Carsner (*5*), in his early studies of curly top, showed that beets affected with a mild form of the disease were neither immune from nor more resistant to severe forms of the disease. Giddings (*7*) has recently shown that previous invasion of sugar-beet plants by less virulent strains of virus does not protect against other strains of high virulence superimposed upon the first and, further, that strains of high virulence do not prevent strains of low virulence from becoming established. It has not been possible to study the matter of simple strain protection in tomatoes, because all of the known strains of the curly top virus either fail to infect or else are extremely virulent on this host. Yet, after tomato plants become immunized, a certain degree of cross-protection between virus strains becomes effective. This type of cross-protection in immunized plants results from a specific reaction in the plant in which the immunity originates. If it were simply a mildly reacting strain of virus protecting against a related, more virulent strain, then the immunity against one strain of curly top virus would be expected to protect the plants against all others, because the strains of this virus are certainly very closely related. Furthermore, the mildly diseased condition of the immunized plants and their resistance to reinfection can hardly be explained on the basis of the presence of a mildly reacting strain of virus, since, in the experimental work here reported, the tomato plants were immunized against strains of high virulence that persisted in the plants without change.

The demonstration that a period of time is required for tobacco plants inoculated by means of leafhoppers to reach a condition in which they can confer passive protection on other plants is evidence that the condition of acquired immunity involves the production of protective substances within the plant as a result of its being infected with the virus.

The fact that tomato plants of cultivated varieties, which rarely initiate the reaction leading to recovery and acquired immunity, can be provided with this condition when they are grafted with immunized tobacco plants is difficult to explain other than on an immunologic basis. The condition eventually reached in such plants suggests a type of passive immunization which partly protects the plants during the early stages of disease and thereafter incites an active production of protective substances by the recipient plant. Such a reaction differs from the usual conception of passive immunization in the field of animal pathology, but Kolmer and Tuft (*9, p. 34*) suggest a similar phenomenon in the following statement: "Acquired immunity occurs in two distinct forms: (1) *active* and (2) *passive*. A mixed form may exist, brought about by a combination of factors necessary for the development of the other two."

SUMMARY AND CONCLUSIONS

Tomato plants of cultivated varieties become severely diseased when infected with the curly top virus either by leafhopper inoculation or by grafting with other tomato plants that have been infected by means of leafhopper inoculation. If, on the other hand, they are grafted with Turkish tobacco plants in which recovery, or the reaction leading to recovery, has occurred, the tomato plants acquire an immunity similar to that observed in recovered tobacco plants. Since the tomato plants rarely show any tendency to initiate the recovery reaction, this transfer of immunity may be considered a type of passive immunization in plants, although it differs from ordinary passive immunization in animals. Actually, this immunization may be passive only in the sense that certain substances are transferred from the immunized tobacco plants that protect the tomato plants to some extent during the early stages of disease and incite the production of like protective substances in the recipient plants. The retention of the acquired immunity in the tomato plants through many successive propagations, after it once becomes established, suggests that an active process is in operation.

By the use of Turkish tobacco, which always recovers from curly top, it was possible by graft transfer to immunize tomato plants against individual strains of the curly top virus. Immunized tomato plants varied widely in vigor and symptom manifestation according to the virus strain used. Clonal plants propagated from the immunized plants also showed the same wide variation of response, ranging from vigorous, at times symptomless, plants to those of low vigor with conspicuous curly top symptoms. These variations among tomato clones immunized against different virus strains were fairly consistent, and for the most part seemed attributable to the virus strains rather than to individual plant variation.

Tomato clones immunized by single virus strains, when tested by reinoculations which superimposed different virus strains, showed high protection against some strains and less against others. These reactions indicated a definite specificity of immunization. The protection given by one virus strain might be equally effective against many strains, but occasionally the protection was found to be relatively low both from the standpoint of numbers of strains and degree of injury caused by individual strains. These results were confirmed by performance of clones of immunized tomato plants grown in the field under heavy natural exposure to curly top.

Since there are no known strains of curly top virus that react mildly on tomato, it has not been possible to determine whether a mildly reacting strain in a nonimmunized tomato plant would protect against the more virulent strains. However, in tomato plants immunized against and carrying virulent strains of the virus, cross-protection of a kind has been demonstrated. This differs from ordinary cross-protection in that (1) the mild symptoms of the immunized plants are not due to the presence of a virus strain of low virulence and (2) the degree of protection varies from complete to slight, depending on which strain of virus is carried by the immunized plants or which strain is used for reinoculation of the immunized plants. Since all of the virus strains used in these studies are virulent on tomato and since the virus strains have remained stable over a long period of testing on

numerous differential plant hosts, it seems most certain that the recovery and acquired immunity from curly top cannot be explained by strain antagonism.

Experiments dealing with graft transfer from immunized plants to healthy plants, in comparison with similar transfer from nonimmunized to healthy plants, led to the conclusion that recovery and acquired immunity from curly top are not correlated with virus invasion of immature tissues.

Recovery and acquired immunity from curly top in tobacco and tomato seem to differ fundamentally from similar phenomena described for other plant viruses and seem closely comparable to reactions known for animal viruses. The experiments that deal with passive transfer of protective substances and the time element involved in the recovery process, during which the protective substances are generated, support this position. Furthermore, the specificity among virus strains, with respect to their immunizing potentialities and the degree of protection conferred either actively or passively, corresponds with results reported for animal virus strains. The persistence of virus in the recovered plants no longer excludes these plant reactions from the field of immunology, as more evidence is obtained that immunity from certain animal viruses may actually depend on the continued presence of the causative virus.

This study on plant reactions to curly top virus has given the following findings: Regularly occurring recovery in tobacco; acquired resistance of recovered plants to injury from reinoculation; persistence in recovered plants of curly top virus not lessened in virulence; evidence of a time factor involved in the reactions leading to recovery; evidence that this recovery and acquired resistance does not result from invasion of embryonic tissues; proof of transfer by grafting of the acquired condition of tolerance and resistance from a recovered plant, not only as an intraspecific transfer (tobacco to tobacco) but as an interspecific passage (tobacco to varieties of tomato that very rarely initiate the recovery reaction); and, finally, evidence of the striking specificity exhibited by different strains of the virus. This whole range of experimental evidence clearly indicates that the phenomena are immunologic in nature.

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